

The Miracle Drugs

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THE DISCOVERER OF PENICILLIN—DR. ALEXANDER FLEMING

THE MIRACLE DRUGS

by
BORIS SOKOLOFF, M.D., Ph.D.
Director of Research,
Florida Southern College



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1

Two Ideas: Pasteur vs. Ehrlich

ON DECEMBER 27, 1892, a great celebration took place in Paris. The vast hall at the famous Sorbonne was crowded far beyond capacity. The Republic of France and the scientists of the world were celebrating the seventieth birthday of Louis Pasteur. Ministers and ambassadors, members of the French Chambers, foreign delegations, and eminent scientists came to pay their respects. Pasteur's collaborators, among them Pierre Emile Roux, Jules Bordet, Charles Chamberland and Elie Metchnikoff occupied seats of honor. Pasteur, a short, bearded man with gray hair and tired features, was deeply moved. Almost too ill to speak, he choked with deep emotion when an elderly man, as old as Pasteur himself, arose to address the assemblage. This distinguished-looking figure with wavy hair and heavy sidewhiskers was Dr. Joseph Lister, the famous English surgeon.

"You have raised the veil that for centuries cloaked the infectious diseases," he said to Pasteur.

With an effort Pasteur got to his feet and embraced him.

Pasteur could not deliver his own address. His voice, once powerful and commanding, was now thin and shrill. His son read his paper.

' Whether or not our efforts are favored by life, let us be able to say, when we approach the great goal, 'I have done what I could ' "

This dramatic scene was symbolic. Here were two men who had been fighting shoulder to shoulder for the same ideas. Both had been persecuted and criticized yet each had triumphed. One had laid the foundation for the science of bacteriology, for Pasteur is the acknowledged father and creator of this branch of science, a man whose service to humanity is beyond appraisal. Lister had founded the applied science of antiseptics, for he was the first to advocate the use of disinfectants for wounds and infections. While the progress in bacteriology was spectacularly dramatic, the science of antiseptics developed slowly and painfully. Its achievements were sometimes superficial and often controversial in nature.

In 1867 Lister, then Professor of Surgery at Glasgow University, published in the *British Medical Journal* (vol. 2, p. 246, 1867) his paper, revolutionary for his time, entitled 'On the Antiseptic Principle in the Practice of Surgery'. In his introduction Lister wrote 'When it had been shown by the researches of Pasteur that the septic property of the atmosphere depended not on the oxygen or any gaseous constituent, but on minute organisms suspended in it, which owed their energy to their vitality, it occurred to me that the decomposition in the injured part might be avoided without excluding the air, by applying as a dressing some material capable of destroying the life of the floating particles'. He then described in detail how he had succeeded in keeping wounds clean and uninfected.

As a great surgeon and a man of vision, he was appalled by the high mortality rate of patients who survived surgery but then died from blood infections. Inspired by the work of Pasteur, he had determined to apply the discoveries of the French scientist. He accepted without reservation the pos-

tulate that the infection of wounds and fractures was caused by microbes, although at that time little was known about disease producing germs. He reasoned if only the mysterious agents of infection could be kept out of human flesh, the healing of wounds could proceed without delay or painful complications.

But how to achieve this goal? How keep the wounds free of pathogenic bacteria? One day several of his friends brought to his attention a newspaper article about the effective use of carbolic acid for the disinfection of sewage in Carlisle. Lister was inspired. If carbolic acid could prevent putrefaction it might also serve as an antiseptic for wounds. So he decided to use this chemical in his surgical procedure.

To make his technique more effective and to make certain *no microbes would contaminate the wounds*, Lister applied carbolic acid solution on a wide scale. Not only the skin of the patient, but instruments, sponges, ligatures and dressings were washed and soaked in this solution, the hands of the surgeon also received a carbolic acid bath before the operation. This technique which today would be considered quite extreme and not entirely rational, brought immediate results. The number of postoperative infections and consequently the deaths in Lister's practice, dropped miraculously from almost 50 per cent to 10 per cent. With every new surgical case in which antiseptic measures were taken, Lister became more enthusiastic. He advocated the use of the carbolic acid solution in surgery so convincingly that soon many other surgeons accepted his technique. Carbolic acid in surgery became the vogue and remained so for more than two decades. It was largely used in infections, and an attempt was even made to use it as an internal antiseptic by injection under the skin.

Lister had not been the first surgeon to use carbolic acid in the operating room. A few years earlier, Dr. Georges De

clat a French surgeon had explored its antiseptic properties not only in surgery, but also in the treatment of many local infections of the skin and throat. He had even published a treatise on this subject *Nouvelles Applications de L'Acide Phenique en Medicine et en Chirurgie, 1864*. But it was Lister with great drive and almost fanatical faith plus his dynamic capacity for persuasion who was able to establish this technique as an indispensable step in every surgical operation. To him must go the credit for originating that branch of medical science—antiseptic surgery.

In spite of the fact that carbolic acid was effective in surgery, its limitations as an antiseptic gradually were recognized. As progress was made in bacteriological technique it was soon proved that carbolic acid which in a 3 per cent solution is to some extent harmful to living tissues was unable to destroy all bacteria. It became evident that this preparation could hardly be considered an ideal antiseptic even for external use.

Many other chemicals and compounds were suggested, tested and subsequently applied with varying degrees of success. Yet none met the requirements of an ideal antiseptic. While in a very low concentration they were often detrimental to microbes, many were also harmful to some extent to living tissues. Although many of these antiseptics are widely used even to the present day, all as a matter of fact are poisonous to the human body in the concentration necessary to kill the microbes. For this reason none can be injected into the system as an internal antiseptic. Yet as years went by, medical scientists became more and more eager to find a substance that would truly prove to be the efficient internal antiseptic.

At the time Lister was laying the foundation for the use of antiseptics in medicine and had actually taken the first

step in the direction of chemotherapy, the scientific world of Europe was in a state of confusion. The theory of spontaneous generation still dominated the minds of many leading medical men, who defended it with ferocity and anger against that small group of pioneers headed by Lister, Pasteur, and Tyndall. As late as 1867, E. Hallier, of Jena, tried to prove that molds could be transformed into bacteria, using his observations as evidence of the spontaneous-generation theory. Only the work of Pasteur, De Bary, and Robert Koch dealt the final blow to this relic of religious superstitions which originated in the dark days of the Middle Ages.

Once science was freed forever from the influence of that theory, the progress of bacteriology proceeded with remarkable swiftness. Now that science had grasped the technique of the isolation and cultivation of bacteria, the attention of Pasteur and his associates was focused on the fundamental question: How can the organism defend itself against the invading germs? Pasteur, by now an old man, interested himself in the recovery of various animals from infectious diseases. How could one, he wondered, increase the resistance of the organisms of animal and man alike against the disease-producing germs? His first experiment in this direction was to inject chicks with old, no longer virulent cultures of chick cholera, against which the organism could defend itself more successfully. The infected fowl became only mildly ill and soon recovered from the infection. He labeled such a tired culture an attenuated virus. At once he realized the principle of this reaction, namely the setting up of an immunity against the germ. These tired cultures he called vaccines. A few years later he elaborated the attenuated virus against rabies.

But how does the organism defend itself against invading germs? What is the principle of immunity? What is its bio-

logical nature? Pasteur himself did not answer these questions. But his disciples, Bordet, Metchnikoff, Roux, and Besredka gave them great attention.

Investigating the common water flea *Daphnia*, Metchnikoff came upon a peculiar phenomenon. Injecting into the body of this flea some fungus like germs he observed that the organism of the flea defended itself by sending small, mobile cells to attack the invaders. He continued the same experiments with mice and frogs and found there the same savage war between the invaders—the germs—and the defenders—the mobile cells of the organism. If the bacteria were not sufficiently virulent and numerous they fell victim to the defenders and were actually devoured by the mobile guards of the body. Metchnikoff called these minute cells of the organism *phagocytes*. Gradually his theory of *phagocytosis* became accepted as the correct interpretation of the general nature of the defense mechanism of the human and animal body.

Following the fundamental ideas of Pasteur and Metchnikoff their disciples visualized that the fight against infections called for measures which would destroy the invading germs by exploiting the natural defense mechanism of the body. They firmly believed that medical science should follow the paths of research indicated by these natural forces which dominated and controlled the human organism—forces which could not be changed and should not be neglected. Increasing the natural resistance of the organism—preparing various types of vaccines—extending immunization against as many types of bacteria as possible—that was the research procedure which the disciples of Pasteur charted and followed faithfully.

As early as 1877 Pasteur observed that some bacteria were capable of inhibiting the growth of the anthrax germ. The notion that some bacteria commonly present in the human body but harmless to their host might produce substances

which possessed the property of destroying disease-producing germs appealed to the disciples of Pasteur. Numerous attempts in this direction were made at the Pasteur Institute with little practical result. In spite of their failure to isolate an antibacterial substance from molds and bacteria, the idea persisted with the workers of the Institute that some day such a substance, harmless for human beings but detrimental to germs, might be discovered. But they never expressed the conviction—they never had any such conviction—that such a substance would be capable of destroying the germ directly when introduced into the blood stream. They visualized this anti-germ material as one which would cooperate with the defense forces of the organism and strengthen them.

Quite another approach to this problem was attempted by the scientists in Germany. Paul Ehrlich and his followers firmly believed that a "magic bullet," which would kill each and every germ, could be discovered. In accordance with his famous side-chain, or receptor, theory of immunity and his belief in the chemical nature of the phenomenon of immunity, Ehrlich stubbornly and persistently searched for a "magic bullet," an antibacterial chemical compound. He gave little consideration to the natural defense forces of the organism as such.

The sulfa drugs are the children, the creations of Ehrlich's school. The natural antibiotics, penicillin and other substances extracted from molds and bacteria, reflect to a great extent the general idea of an antibacterial substance which dominated, and still dominates, the work at the Pasteur Institute. But the paradox concerning these two conflicting ideas on the proper method of fighting germs, is that neither concept has quite been realized. The sulfa drugs are not "magic bullets" which kill the germs directly, nor do the antibiotics, which are isolated from molds and bacteria, increase the natural resistance of the organism.

The basis of present-day chemotherapy lies somewhere between these two concepts. As frequently in scientific research the conflict between two different, and often opposing ideas brought in the end a remarkable success to which both of these scientific schools contributed greatly. As a matter of fact, it is still not established precisely how sulfa drugs, penicillin and other antibiotics destroy germs. Nevertheless, the fact remains that they are truly miracle drugs.

2

The "Magic Bullet"



THE BIOLOGICAL LABORATORIES in Germany at the end of the last century presented a curious picture. At the University of Heidelberg, and at the much more modern University of Hamburg, one found the same strange situation. In fact, all over Germany scientists were deeply engrossed in the investigation of dyes. Everywhere, whether in the famous laboratory of Professor Otto Butschli or in some obscure hospital laboratory, investigators were concentrating on the study of the effect of dyes on dead and living tissues. Numerous azo-dyes were being tried in various combinations for the differential staining of tissues, as well as for their therapeutic effects.

This concentration was not entirely accidental. It was a logical by-product of the enormous growth and expansion of the I. G. Farben Industries. Obviously, this powerful organization stimulated and encouraged these investigations to further their commercial activities. The scientific articles published in Germany during the first two decades of this century contain hundreds of papers dealing with various dyes and stains and their application to experimental cytology and medicine.

When Paul Ehrlich entered the medical school of Breslau, histology, the science of tissue structure, was receiving great attention in Germany. From his first student years the intrinsic nature of living cells attracted Ehrlich's attention. This was the period when the mechanistic theories were gaining acceptance and young students, biological and medical alike, were seeing living cells and organisms in terms of chemical "machines." Biological chemistry, still rudimentary, inflamed their imaginations by the exciting prospects which the new discoveries had opened. "The living cell is nothing but a small chemical plant," the young Ehrlich proclaimed. At that time he was, properly speaking, much more interested in cytology and histology than in medical science. Instead of learning about the symptoms and diagnosis of various diseases, he gave his attention to the technique of tissue dyeing. The aniline dyes fascinated him. Even then, young as he was, and ignorant of bacteriology, he intuitively felt that there were vast possibilities and a great future in the application of dyes for therapeutic purposes.

Pasteur had already made his important discoveries when Ehrlich was still playing with dyes, having no clear idea to what purposes they might be put, except for the staining of living and dead tissues. Along the same lines, his doctor's thesis was entitled: "Contribution to the Theory and Practice of Tissue Staining."

He was past thirty, restless because he had not yet found his creative bent, when quite by accident he witnessed Robert Köch's demonstration of his newly discovered tubercle bacillus. Koch's report made a deep impression on Ehrlich. In fact, this marked the turning-point in his life. At last he saw the road opening before him and what he must do. To fight germs with his beloved aniline dyes, to make these dyes effective against germs—at last his destiny was clear. His plans for future work were vague and not yet crystallized, but he had



LOUIS PASTEUR

Acme



PAUL EHRLICH

Acme

an inner feeling that he would soon be following a direct line to his goal. He decided to work with Koch and to learn more about the disease causing germs. It was he who taught Koch to stain the tubercle bacillus properly. In return, he gained a grasp of the newly born science of bacteriology.

Ehrlich was quite exhilarated by the fact that he had succeeded where Koch had not. It was a small thing, this staining of the tubercle bacilli, and yet he was able to prove to the great bacteriologist that he was wrong to assume that the tubercle bacilli could not be stained. Using the aniline water gentian violet, he at once prepared numerous slides of the same bacteria, which appeared a brilliant shade of violet under the microscope. Here, at least his faith that there must be an affinity of the cell of the bacterium for some specific dye, found convincing proof.

For several years he was associated with the Institute of Robert Koch in Berlin. The work there was absorbing, but it had no direct connection with his obsessive idea of using azo-dyes for therapeutic purposes.

Now and then he snatched a little time to make a few experiments with the dye of his choice, methylene blue. Impressed by the fact that when injected into the blood stream this dye had an affinity for living nerve cells and would stain them blue, Ehrlich embarked on a rather bold experiment. 'Why not treat pain with this dye?' he asked himself. He promptly decided to inject the blue dye into the blood streams of patients suffering from pain caused by tumor growths. The injections were well tolerated, but they gave no relief from the pain.

However, from this failure a new idea gradually crystallized in his mind. 'What type of azo-dye should be used to kill germs?' he asked. Certainly not the dye which stained all living cells of the organism—that was now clear. One must find a dye which would stain living germs, but leave un-

touched the tissues of the human body a selective dye with a highly pronounced affinity for the germs. Thus his plan of attack on the problem was prepared but many years passed before he was in a position to work in this direction. In the meantime he became engrossed in the investigation of immunity.

He had an analytical mind. He would develop some theory and elaborate it precisely. To him these theories were like houses he planned to build; he wanted to blueprint every detail far in advance. His exactness in calculation was absolute and to some of his colleagues even appalling. His approach to scientific problems was the opposite of Pasteur's. The French scientist never depended on his own preconceived theories but conducted his investigations along the lines of some working hypothesis which he never fully accepted or rejected until his experimentation provided sufficient evidence. But Ehrlich's mind often stubbornly and persistently defended a theory which he had conceived long before the experimental data was accumulated.

His theory of immunity was the work of a brilliant intellect. Small wonder that it dominated bacteriological thinking for the next twenty years.

His side-chain theory is not easy to explain for while it is impressive in the perfection of its logical structure it is full of theoretical speculations. Why should the organism become resistant to certain bacteria? How is an immunity toward some types of bacterial toxins set up in the tissues of the body? Ehrlich tried to answer the same questions which Pasteur had left unsolved at his death. When Ehrlich had been a young student he had regarded the living cell as a minute chemical plant. He remained faithful to this concept. On this assumption he built his theory of immunity which today appears to be somewhat oversimplified and far from convincing.

The living cell is conceived as consisting of a central chemi

cal nucleus, which Ehrlich called the *Leistungskern*. On this nucleus, which is more or less stable, the function of the cell depends to a great degree. Through its side chain of atoms this chemical nucleus enters into relationship with various chemical substances. Thus we may imagine the chemical central nucleus as, for example, the central carbon ring in aspirin (salicylic acid) in which the hydrogen atoms represent side chains.

The cell, therefore, is considered an independent chemical unit, different from others because of its *Leistungskern* but, with the help of its side chains, in constant intercourse with various materials and constantly getting rid of waste products. In order to enter into similar relationship with toxins of bacteria, the living cell unites with the antibodies substances. In some instances the cell assimilates these substances harmlessly. In other cases, it might be injured in the process. That, in brief, is the essence of Ehrlich's theory of immunity, which made him known to every bacteriologist of his time.

In 1901 Ehrlich, forty-seven years old, was the head of his own laboratory at Frankfort on the Main, the seat of the I. G. Farben Industries. He was now very close indeed to the center of production of his beloved dyes. Yet his idea of the "magic bullet" was as far from realization as it had been ten years before. His laboratory was small and poorly equipped, although it carried the rather imposing title of "Royal Prussian Institute for Serum Testing."

However, Ehrlich was not a man to lose courage. More than ever, he was convinced that he would some day discover an ideal drug to kill germs. Meanwhile, he was busy with the work of testing serums. He soon succeeded in the development of a precise method of testing antiserum for diphtheria. This was of considerable practical importance.

The minds of men sometimes operate in unpredictable ways. This man of intellect often acted on impulse, as though

following some inner voice As early as 1880, more than twenty years before, the agent of malaria had been discovered by Alphonse Laveran, who had described the organism later called *Haematozoa malariae* Soon similar organisms, also parasitic unicellulars which caused sleeping sickness in man, as well as dangerous diseases in horses, were described by the workers at the Pasteur Institute Ehrlich knew about these discoveries but, at the time paid no particular attention to them When he learned that Laveran was able to transfer *Trypanosoma equinum*, the parasite which affects horses, into mice he became quite excited Here is the organism with which we must work he explained Let us transfer the trypanosomes to mice and treat them with dyes Thus, in 1902, began a new period in his research activity He was working with dyes in search of the 'magic bullet.'

The organism with which Ehrlich was now working was originally discovered in South America in dying horses This aggressive unicellular with the beautiful small tail was killing horses by the thousands for it caused a sickness known as *mal de caderas* When transmitted to mice the infection became as grave as in horses It killed the animals in fifteen to twenty days if not earlier To Ehrlich, the path of the investigation was now clear Together with a Japanese doctor, K. Shiga, he began the task of finding the therapeutic drug among the hundred various dyes in his laboratory One after another they tried azo-dyes and watched the mice infected with trypanosomes. The mice treated and untreated alike, died from the terrible infection The symptoms were not affected by the injected dye, nor did it delay the deadly action of the germ

For two long years they tested about four hundred dyes, only to find that not one had any therapeutic effect upon the sick mice Their deaths came as promptly as though nothing had been administered to save them

Ehrlich was not disappointed; he was not a man to lose confidence. His theory must triumph. "Some day," he repeated, "the magic dye will be found."

Suddenly he was seized by a new idea. "I know now what is wrong with our dyes," he said to Dr. Shiga. "They have no specific affinity for the trypanosomes. We must make a dye which will have a specific action upon them. Why not improve the dye by adding a sulfo compound to it?"

This was not an easy task. Ehrlich was obliged to ask the help of the Farben Industries chemists. They promptly supplied him with a red azo-dye containing sulfo group, which was called *trypan red*. This was slightly effective, but not sufficiently so to cure the disease. Some of the mice infected with trypanosomes and treated with trypan red did live longer; some, however, died as promptly as the untreated mice. The experiments were not a success, particularly when Ehrlich tried to treat dogs. The dogs treated with trypan red died even faster than those that had not received the drug.

Ehrlich had lost the first round, but not entirely. He had proved that the azo-dyes containing sulfo group had some therapeutic effect on the agents causing sleeping sickness and *mal de caderas*. Even more important was the fact that his work aroused considerable interest in the medical circles of Frankfort on the Main. There was much discussion of the doctor who was seeking a miracle drug for certain dangerous diseases.

In 1906 a rich widow, Mrs. George Speyer, decided to sponsor Ehrlich's work. She gave him the money to build a large laboratory for his experiments. Thus the George Speyer Institute was founded and put at his disposal. From now on he had everything he needed to work at full speed on the idea which he had been nursing for so many years. There was no more worry about funds for his investigations.

The azo-dyes were a disappointment. He decided that a

new compound must be found. His attention turned to a preparation recently described under the name of atoxyl. It was claimed that atoxyl was effective in treating mice infected with the agent of sleeping sickness. The drug had even been tried on a few African Negroes with some degree of success but due to the toxicity of the drug with a high rate of mortality Atoxyl was actually too harmful for use in medical practice for it was made of a benzol ring with attached to it, a compound of oxide arsenic. The formula itself suggested that the drug must be damaging to human beings.

Ehrlich realized that the compound was of a poisonous nature but he saw in it a seed of potentiality. We may as well modify the drug he announced to his collaborators and make it harmless.

Thus began his famous investigation of arsenic compounds which in the end brought into existence salvarsan. Ehrlich's 606. The researchers hurdled many obstacles and overcame many difficulties. They prepared endless numbers of compounds during the two years that followed his decision to find a harmless and yet potent antibacterial drug consisting of arsenic and azo-dye. Each compound was numbered. And each new sample when tested on the mice infected with sleeping sickness was a failure. Months went by without any hope of success. His collaborators were exhausted by this fantastic hunt for an unknown substance and long before had given up any hope of finding the magic drug. Sometimes a new compound seemed to prove slightly more effective. The course of the infection was delayed in the mice only to prove a few days later that the newly prepared drug was either too toxic or of such minor effectiveness that it was powerless to kill the germs. Ehrlich's fundamental idea was to find a drug which in a single dose injected into the organism would destroy all germs. But one powerful dose was often lethal. Divided into smaller doses the drug might not work at all.

The only man who never lost his courage, who always retained his enthusiasm, was Ehrlich himself. He never ceased seeking to inspire his associates with his own faith. When more than three hundred compounds had been prepared and still there was no visible sign of success, even Ehrlich showed evidences of moral exhaustion. But precisely at this critical moment he received encouragement he had not expected. In 1908, in the midst of his wild search for the "magic bullet," he was awarded the Nobel Prize, to be shared with Metchnikoff. This event seemed to him to be an indication that he was on the right track.

Almost two years of intensive, heartbreaking investigation had passed. Six hundred compounds had been prepared. Each had been carefully tested on animals and each had proved to be of no practical value. Then, quite unexpectedly, came the first glimmer of success. When they began to work on the compound bearing the number 601, the relative harmlessness of the drug and its effectiveness became evident. The drug was still far from perfect, but there was hope that, with slight improvement, it might work.

Shortly afterward, the compound "606" was prepared. Great credit for this discovery should be given to Dr. Franz Bertheim, Ehrlich's associate in this work. A brilliant chemist, he was able to achieve what had hitherto been impossible. The arsenic part of the drug was rendered almost nontoxic.

Yet when "606," later named salvarsan, was tested on the mice infected with sleeping sickness, it did not have the effect which had been anticipated. Some of the mice were cured; others, however, showed no signs of recovery. Altogether, the drug was not very effective in the treatment of sleeping sickness and *mal de caderas* in mice.

A few years before Ehrlich plunged so deeply into his search for an ideal drug, a German protozoologist, F. Schaudinn, working with Dr. E. Hoffmann, had discovered in syph-

ilitic ulcer a microorganism which was identified as a spirochete. At first Schaudinn thought that this microorganism—the germ of syphilis—was not a bacterium, but a unicellular. He called it *Treponema pallidum*. We now know that the syphilitic spirochete belongs to the bacteria group, although it possesses some characteristics distinctly different from those of typical bacteria. Ehrlich was most impressed by the fact—an error on the part of Schaudinn—that the germ of syphilis was of the same class of microorganisms as the agent of sleeping diseases.

The possibility of treating syphilis inflamed his imagination. 'I will be able to save millions of lives!' he shouted to his colleagues. 'Let us try salvarsan on syphilis.' He already knew that the germ of syphilis could be transferred to rabbits. E. Bertarelli had proved this in 1906. Instead of mice Ehrlich ordered large numbers of rabbits and began to infect them with the spirochetes. In this work, Dr. Sahachiro Hata, another Japanese doctor, assisted him.

Four weeks later a dozen rabbits showed evidence of syphilitic ulcers. There was no doubt that the ulcers were very active and full of dangerous spirochetes. Ehrlich and Hata both made the microscopic examinations, to be sure that they were dealing with fully developed infections.

Late in August, 1909, they decided the time had come to test the salvarsan. Both were nervous. Both had the feeling that it was a decisive moment for the whole research idea. If the drug was a failure, would it mean the end of their dreams? They chose three rabbits with the largest ulcers. Dr. Hata injected the drug into the ear vein of each animal, each rabbit received only a single injection.

That night Ehrlich could not sleep. Early in the morning he hurried to the laboratory to examine the rabbits. They were alive. The ulcers also looked different. They seemed dried up, less inflamed.

Dr Hata promptly examined the contents of the ulcers under the microscope. A miracle! They could not find any more spirochetes in the ulcers.

Ehrlich was jubilant, perhaps overenthusiastic. On September 15 he wrote "It is evident from these experiments that, if a large enough dose is given, the spirochetes disappear completely, and almost immediately after the injection. Soon he realized that this was not exactly true, and that some spirochetes remained alive a day or two after the injection of the drug. However, for Ehrlich it was a triumph of the idea which he had defended so faithfully for more than twenty years. His miracle drug, his magic bullet, was discovered at last.

Now he was in a position to make tests on apes. Six years before, Metchnikoff and Roux had reported that they had succeeded in infecting a female chimpanzee with human syphilis. But their method was neither quick acting nor satisfactory. Ehrlich ordered several chimpanzees. When they arrived, he infected them with the syphilitic germ using the pure culture of spirochetes. Several weeks later the animals were affected with typical syphilitic ulcers, almost identical to those of man.

This time Ehrlich decided to give the animals a much larger dose. "Only a large dose can do the trick," he declared. The drug, almost one gram, was injected into the vein of the chimpanzee. Two days later no sign of living spirochetes could be detected in the ulcers. He repeated the same experiment on other chimpanzees with the same gratifying results. A single dose of salvarsan was capable of completely curing the apes who had been infected with experimental syphilis.

The ground was broken for clinical investigations. The salvarsan experiments on animals were so conclusive and impressive that Ehrlich decided to begin tests on human be-

ings In the beginning he asked only a few of his physician friends to treat their patients with the drug But when the first results of the treatment arrived—and they were highly enthusiastic—salvarsan was distributed throughout the profession on a wide scale

Then disturbing news began to reach Ehrlich Several patients had died soon after the injections Others although treated, were not cured The drug, at least in a single dose, was ineffective After one year of intensive clinical investigation, it became clear that salvarsan was not a 'magic bullet' for syphilis Only cases of fresh infection responded in a satisfactory manner Syphilis cases of long duration showed considerable resistance to the treatment with salvarsan. But worst of all was the toxicity of the drug While some persons seemed to tolerate it well others had dangerous reactions often resulting in death

Appalled and distressed, Ehrlich began to work on the improvement of salvarsan Soon he announced a less toxic drug of higher efficiency He called it neosalvarsan or Ehrlich's 914, a condensation product of formaldehyde, sulfoxylate of sodium, and salvarsan It was much less toxic and did not produce the necrosis of the veins which salvarsan often did But even neosalvarsan was far from the "magic bullet" of Ehrlich's dream It helped in syphilis, and it was able to cure certain fresh infections but it was much less efficient in long-standing cases of syphilitic infection Although a valuable discovery, and a great step in the chemotherapy of infectious diseases, it was a disappointment to the man who all his life had visualized a 'magic bullet' which would save mankind at one stroke from its eternal enemy, disease-producing germs

Three years later, on August 20, 1915, Dr Paul Ehrlich died, the shadow of the man who had held so fanatically to

his idea. While he did not achieve the goal to which he was drawn as by some unknown force, he was the creator of the new science of chemotherapy. For he cut new trails through the unknown, and prepared the way for the future progress and success of chemotherapy.

3

Sulfonamides Rediscovered

THE CLIMAX OF Ehrlich's activity was the discovery of salvarsan. It was a victory of chemotherapy, the triumph of the theory he had so ardently propounded. But after the first days of excitement among his associates in the investigation who alongside him had created the foundation for chemotherapy, an inner crisis gradually began to develop in this branch of science.

As years went by and no further noticeable progress in chemotherapy was recorded, the optimism of Ehrlich's day gave way to gloom and defeatism. Had the great German scientist been on the right track in his search for a magic bullet which would kill germs by a single blow yet leave the tissues of the human organism unharmed? Salvarsan was by no means a magic bullet. Its effectiveness against the spirochetes was only slightly higher than that of older remedies used in the treatment of syphilis. Nor was the drug completely harmless to vital organs of the human body, as Ehrlich had hoped.

Inevitably, the question was raised anew: could such a magic drug ever be discovered? could such a drug actually exist? Was not the whole quest for an antimicrobial agent

much more complicated and the interrelationship between pathogenic bacteria and their hosts much more intricate, than the Ehrlich school had assumed? It was pointed out—and with good reason—that Ehrlich's success was limited to the therapy of an infection caused by spirochetes, which are not typical bacteria. No data existed, nor any records of success in the treatment of infectious diseases due to pathogenic bacteria.

More and more the attention of medical scientists turned back to Pasteur's first formulation of the principles of bodily resistance to bacterial infections. Again the work on vaccines and antitoxins became the focus of attention and the center of investigation.

Once more the old theory of microbe exhaustion was revived, reappraised and applied in the discovery of bacteriophages. These mysterious substances were discovered independently by F. W. Twort, an Englishman, and F. d'Herelle, a Frenchman. Dr. Twort was seeking a vaccine against virus. One day in 1915, working with a culture of micrococci, he noticed a strange development. The culture plate containing this microorganism went through a peculiar change. The colony of the germ became glassy and translucent. When the material was transferred to another plate, also containing the same germ, the same thing happened again. Apparently the substance produced by the germ was capable of destroying the germ itself.¹

Two years later, Dr. F. d'Herelle, working in Mexico on an infectious disease of locusts, came across the same phenomenon. He knew nothing about the work of Twort. He found that the filtrate of the feces of patients who were affected with dysentery, and who had recovered from the infection, dissolved the germ of dysentery.²

Further investigations showed that this bacteria-destroying substance is found in the organisms of man and animal alike.

after recovery from infectious diseases. This substance was called bacteriophage, which means "devourer of bacteria." When this peculiar substance was examined under the microscope, it was found to consist of infinitesimal bodies that were not visible under ordinary methods of examination. They ranged in size from eighty microns to hardly ten microns in diameter.

Heated discussions arose about the nature of these "devourers of bacteria." Some expressed the opinion that they were parasitic ultra microorganisms of bacteria, and that they were, therefore, living organisms. D. Herelle himself defended the viewpoint of the living nature of bacteriophages.³ He believed that the "devourers of bacteria" greatly helped the organism in recovering from infection. The appearance of the bacteriophages, which possessed such extraordinary power to destroy germs in the infected organism, he reasoned, must result in recovery from the infection. Bordet, however, argued that this substance was not a living organism, but a 'lysogenic' factor derived from the bacterial cells.⁴

Whatever the nature of the bacteriophages, the fact remained that they were present in the intestinal tracts of man and animal after the infection had subsided and that they actually did destroy the bacteria. Thus, they were an indispensable part of the defense mechanism of the organism, helping to resist invading germs.

During the fifteen years following this discovery the most intensive investigations were pursued on this subject in various laboratories, both in the United States and abroad. It was hoped that the bacteriophages might be used in the treatment of infectious diseases. As a matter of fact, many such attempts were made, with little success. Bacteriophages were used for the treatment of blood infections caused by certain germs, and for local infections, but the results of the

therapy were far from being either convincing or encouraging.

In spite of the fact that bacteriophage therapy has played a very insignificant role in the fight against microbes, and the practical application of this discovery amounted to almost nothing, the theoretical interest was enormous, and overshadowed every trend in the search for a magic drug. For two decades the action and nature of the defense mechanism of the organism against invading germs was the chief topic of investigation and of avid attention on the part of medical scientists. The search for a magic drug appeared to be abandoned.

A quarter of a century had passed since the discovery of salvarsan, years which brought little hope or encouragement to those who had participated in the momentous work of Ehrlich. The year 1935 found chemotherapy at a standstill, without any prospects for future progress. In that year rumors began to circulate in the medical circles of Germany regarding a remarkable antimicrobial substance which had been discovered at the laboratories of the I. G. Farben Industries, in Frankfort on the Main. It was said that the investigation of this drug had been carried on for two years and the results so far obtained surpassed all expectations.

In the June, 1935, issue of the German medical journal⁵ appeared a three-page article, entitled "Chemotherapy of Bacterial Infections" by Dr. Gerhard Domagk, then director of the Institute of Experimental Pathology of the Farben Industries. The article was extremely conservative as to conclusions. Dr. Domagk reported briefly that a new antimicrobial drug had been investigated in the test tube and on animals with satisfactory results. Mice infected with a deadly germ (blood-dissolving streptococcus) were cured by this drug. The drug was not toxic and the animals had survived relatively large doses.

The drug was named *prontosil* and its chemical structure was 2', 4'-diamino-azobenzene 4 sulfonamide-HCl. Prontosil became the father of many other sulfonamides, varying in chemical structure but retaining the nucleus of the original drug. The most striking fact of this newly discovered drug was that, actually, it was not new at all, but had been *rediscovered*.


As a matter of fact, this compound had been synthesized twenty-seven years earlier. Dr. P. Gelmo, a chemist of the Farben Industries, had prepared a certain quantity of this remarkable drug in 1908. The sample of prontosil had been placed in the safe of the laboratory and disregarded until 1933. Dr. Domagk did not explain either the reason for the delay in investigating prontosil for microbial properties or why the drug had at last been brought from obscurity. Apparently pure coincidence had played a role in rescuing Dr. Gelmo's discovery from oblivion.

In frantic efforts to revive the idea of the 'magic bullet,' the administration of the Farben Industries had decided to check the inventory of the therapeutic azo compounds prepared at the time of Ehrlich's fame. Existing records show that this drug had never been submitted to Dr. Ehrlich for investigation and testing and had simply been lost in the hundreds of azo-dye samples produced.

In his article Dr. Domagk made the point that the antiseptic properties of azo-dyes were actually reduced by the addition of the sulfonamide group when tested *in vitro*, in the test tube. But paradoxically enough prontosil was much more active than the azo-dyes *in vivo* in animals, moreover, the azo dyes were toxic and killed the animals.

As soon as the news of this discovery reached the workers in France, England, and in this country, intensive investigations were undertaken to discover the active properties of prontosil. Drs. J. Trefouel, F. Nitu, and D. Bovet of the

Pasteur Institute of Paris were the first to show that para aminobenzenesulfonamide was more effective than diamino azobenzenesulfonamide⁶ The chemical structure of the drug which was named in this country "*sulfanilamide*," is

NH_2  SO_2NH_2 and was accepted as such by the Council on Pharmacy of the American Medical Association

Immediately clinical investigations were begun at the Infants and Childrens Hospital in Boston and in other hospitals here and abroad In the meantime, in 1937, Dr Domagk published a new and more complete article on the same subject, entitled Further Studies on the Chemotherapeutical Action of Sulfonamide Compounds on Bacterial Infections⁷ This paper furnished considerable experimental and clinical evidence in support of the remarkable activity of the drug against gonococci the germs which cause venereal disease, gonorrhea and hemolytic streptococci At that time three different compounds three types of sulfonamides had already been prepared by the Farben Industries

Overnight the drug became famous Unknown to the medical profession at large in this country until 1937 it captured the attention of doctors at once by its effectiveness in many infectious diseases In addition to the original compound known as sulfanilamide, many other sulfonamides were discovered among them sulfapyridine sulfathiazole, sulfamerazine, sulfamethazine, sulfaguanidine and sulfadiazine The last named was developed by Dr Richard O Robin and his associates at the Stamford Laboratories of the American Cyanamid Company in 1940⁸ More recently a new compound known as gantrisin (3,4-dimethyl 5 sulfanilamido-isoxazole) was developed It is considerably more water soluble than any of the older compounds and appears to be equally effective therapeutically (Kirby) Particularly popular now are so-called triple sulfonamide mixtures which re

duce the incidence of injury to the kidney. They are considered as a real advance in this therapy.

ANTIMICROBIAL ACTIVITY OF SULFONAMIDES

Man lives in a world of microbes. Bacteria are everywhere in the nasal cavity, on the gums and tongue, on the skin. Some of them are harmless and rarely cause infection. But among the masses of microorganisms which surround us, there are some which are capable of producing infections often of a dangerous nature. Most of the infectious diseases from which modern man suffers are actually caused by bacteria which are not truly pathogenic but rather opportunistic. They may exist for weeks or months without causing any harm. They live in a state of inactivity waiting for an opportunity to invade the tissue and blood stream of the organism. The chance may come in the form of a decaying tooth, a small wound, or a cold.

When the bacteria invade the tissue, there is a strong local reaction. The tissue defends itself against the invader. A sharp fight goes on between the white cells, the defenders, and the bacteria, which multiply rapidly and try to extend the area of invasion. As a result of this deadly fight, pus forms, which consists of numerous white cells and the bodies of dead or living germs.

In the great majority of infections the offenders are the bacteria of the group known as the *pyogenic* or pus forming cocci. Among them the most prominent and aggressive are *Staphylococci*, sometimes called *micrococci*. *Staphylococcus* was first isolated from infected wounds and described simultaneously in 1879 by Koch and Pasteur. These microorganisms are the most frequent causes of boils, carbuncles, and other local infections. But they are also capable of penetrating the blood stream to cause grave and often deadly blood infections. Among them the most noted aggressor is

Staphylococcus aureus. It is a very small, round organism either appearing as individual cells or forming grapelike clusters. The other member of the same genus, *Staphylococcus albus*, is much less aggressive and therefore less pathogenic. However, it may cause skin infection, or even a blood infection.

Among the pyogenic cocci one finds a large group of bacteria which induces grave infectious diseases. They are called streptococci, or chain cocci, owing to the fact that they form chainlike gatherings. In 1879, Pasteur, discussing the properties of his new discovery, staphylococcus, indicated the presence of chainlike microorganisms in some infections.⁹

In 1881, L. Ogston, the English bacteriologist, described these bacteria in greater detail, and emphasized the difference between staphylococci and streptococci.¹⁰ The genus of streptococci is very broad and can roughly be divided into two groups, according to their effect upon the red blood cells. One group embraces the hemolytic streptococci, in which species the germ is capable of splitting, or dissolving, red blood cells and thus inducing anemia in human beings. The hemolytic streptococci are responsible for such diseases as infection of bones (osteomyelitis), infection of the brain cover (meningitis), scarlet fever, tonsillitis, childbed fever, and many skin infections. The nonhemolytic group does not affect the blood of man. Most nonhemolytic streptococci are harmless bacteria, but there is among them one aggressive organism which often causes dangerous infections of the heart valves. This germ is called *Streptococcus viridans*.

Other members of the cocci family are: pneumococci (the bacteria causing infection of the lungs), meningococci (responsible for the infection of the brain cover), and gonococci (the agent of gonorrhea), as well as others which play a smaller role in the infectious diseases of man.

The largest portion of the infectious diseases from which

modern man suffers is caused by pathogenic cocci, which belong to the group of "Gram positive" organisms. This mysterious sounding term merely means that the bacteria in this group can be stained by the method elaborated by Hans C. J. Gram. When stained with gentian violet dye (aqueous solution) the bacteria retain the color. Bacteria which do not retain gentian violet are designated "Gram negative." This division is neither scientific nor strictly accurate, but it is in common use in medical practice.

The sulfonamides have demonstrated their strongest activity against the Gram positive microorganisms. Thus, as a general rule, *sulfonamides are very effective against Gram positive germs, and inactive against Gram negative organisms.* However, these drugs are not active against all Gram-positive, nor are they inactive against all Gram-negative bacteria. There are numerous exceptions to this general rule.

The most striking feature of sulfonamides is their destructive activity against the blood splitting streptococcus. This fact was first observed by Dr. Domagk and served as a point of departure for all further investigations on sulfonamides. When a small amount of sulfanilamide is added to a plate containing the culture of blood splitting streptococci, the germs are unable to grow, and gradually perish. An even more striking reaction occurs when the drug is administered to a mouse or rat. Suppose an animal is infected with the deadly germ, and several hours later given the drug by mouth or injection. Even if he had already become ill, and the infection had spread to his blood stream, the drug can and does save him from death. Unfortunately, sulfonamides are less powerful against staphylococci, although active enough to prevent or to control infections which are not advanced or virulent in animals. Hence, if the drug is given to the animal at the same time he is infected with the germ

the infection may never develop. This is termed 'protection against the infection'.

Sulfonamides are also very destructive to the other cocci, such as pneumococci, meningococci and particularly gonococci. The destructive effect of the drug on gonococci is truly remarkable. When a mouse is infected with this germ, two or three drug injections are sufficient to destroy all the germs floating in the blood stream of the animal, and thus the infection is arrested almost immediately.

Though sulfonamides have been shown to be active mostly against Gram positive bacteria, it was proved that the drug is also detrimental to the germ of dysentery, *Shigella dysenteriae*. This germ, which was discovered by the Japanese doctor K. Shiga in 1898, belongs to the Gram negative group of bacteria.

Sulfonamides are powerless against the germs of syphilis, tuberculosis, and typhoid fever, as well as of many other infectious diseases.¹¹ They are also inactive against the viruses.

One question which interested scientists from the earliest discovery of these remarkable drugs is how they actually exert their destructive power upon the germs. Do they kill the germ directly, as some antiseptics do? Are the sulfonamides the "magic bullet" of Ehrlich which was supposed to kill all the germs at one blow? Although the exact mechanism of action of the drug is not yet defined, all the indications are that the drug *does not kill the germ directly*. It is not a bactericidal substance. Sulfonamides act on germs indirectly. They slow down the growth of bacteria and impede their multiplication. They attenuate their life and activity. Physiologically, the action of sulfonamides can be interpreted as an interference with the normal nourishment of the bacteria by the construction of a chemical wall which deprives them of nutrients essential for their existence and

propagation. By imposing on the bacteria a sort of involuntary starvation, sulfonamides exhaust the vital forces of the germs, and make them powerless to combat the defensive forces of the human organism.

What are these essential nutritive substances? The opinion has been expressed based on certain observations that the sulfonamides interfere with the consumption of oxygen by the susceptible bacteria.¹² On the other hand, it was also shown that sulfonamides prevent one of the factors of vitamin B complex from being properly utilized by the bacterial cells. This vitamin is known as para aminobenzoic acid. It seems that para aminobenzoic acid is essential to bacterial physiology. It plays an important part in the respiratory system of the bacterial cell. When this vitamin is deficient in the nutritive medium, the bacteria either do not multiply at all, or their growth is slowed down considerably. This substance is widely distributed in nature and small but detectable amounts are present in animal organisms.

This fact has been known for some time, but Drs. S. Woods and P. Fildes first demonstrated that there is an antagonistic action between sulfonamides and para aminobenzoic acid. They took mice infected with deadly streptococci and treated them successfully with sulfa drugs. In the next series of experiments they added para aminobenzoic acid to the animals' ration. They again infected the mice with the germs and again treated them with sulfonamide. The treatment was a failure and the animals died. Therefore an excess of this vitamin prevents the sulfa drugs from exerting their antibacterial activity.

Dr. Eugenio Riesz gave a plausible interpretation of this phenomenon. The sulfa drugs, he explained, readily supply electrons to certain substances which are deficient in them. Such a substance is para aminobenzoic acid, essential to the respiratory transport system of bacterial cells. When this vi-

tamin substance extracts the electrons, the sulfa drug is no longer of use to the bacteria, and they starve for lack of oxygen; they are asphyxiated. However, when there is an excess of this vitamin in the system of the animal, the sulfa drugs are unable to interfere with the utilization of this essential substance by bacteria. The sulfa drug might neutralize a portion of the vitamin present in the system, but some still remains unchanged.

Riesz based his theory on the analysis of the chemical structures of various sulfa compounds. According to him, the spatial and structural configurations of the therapeutically active sulfa drugs are such that the electron exchange can take place. Whether or not this theory fully explains the exact mechanism of sulfa drug activity is still unsettled. But one fact is firmly established: the sulfonamides as a rule act indirectly on the germs and therefore they are called *bacteriostatic substances*. They interfere with the normal aggressiveness of germs and inhibit their growth.

BACTERIAL RESISTANCE TO SULFONAMIDES

Bacteria are living cells. As with every living substance, they are able to adapt themselves to unfavorable environments. If a bacterium survived the destructive action of sulfonamide, it might become resistant to this drug. In such a case, even large doses will no longer have a detrimental effect on the germ. As years passed, and hundreds of thousands of people received sulfa drugs, often in relatively small doses, many strains of disease-producing germs became sulfonamide resistant. Today doctors often encounter patients who are infected with germs indifferent to the action of sulfa drugs. According to all symptoms, the patient should be cured by this drug. But when a blood culture is taken, and the germ's susceptibility to the drug is investigated, the test reveals that the bacteria is resistant to the drug. Such reactions occur

particularly often with staphylococcal infections and in gonorrhea

Much attention has been given to this distressing fact by medical men. How do the bacteria develop such a resistance? Some years ago when sulfa compounds were investigated in the test tube, it was found that if the germs are not too numerous they are destroyed successfully and completely. Figuratively speaking, when the drug is fighting a small army of bacteria the victory is assured. But when the army of bacteria is very large and compact the drug works with less efficiency as though the drug was unable to penetrate deeply into the masses of bacteria.¹³

Studying the unusual propensity of staphylococci to develop resistance to the drug Dr. M. Landy and his colleagues arrived at a very interesting conclusion: the bacteria produce an inhibitory substance which permits them to fight against the destructive action of sulfonamides. This substance seems to be closely related to, if not identical with, para-aminobenzoic acid.¹⁴ This observation was later confirmed in regard to other species of bacteria submitted to the action of sulfonamides.¹⁵ The realization of this peculiar ability of the germs to fight against the drug is of considerable practical importance. For there is a danger of giving the patient too small a dose of the drug as was often the case in the past for minor ailments of the skin or for urinary tract infections. The patient may have been cured momentarily of his ailment, but not all the germs destroyed; those which survived became sulfonamide resistant. If this person ever became seriously ill with an infection caused by the same germ the drug would be powerless to give him help.

SULFONAMIDES IN INFECTIOUS DISEASES

Sulfa drugs have been widely used in infectious diseases during the past ten years. There is no doubt that they have

saved the lives of hundreds of thousands of people who would otherwise have succumbed to infection. In meningitis in gonorrhea in some types of pneumonia, in various infections caused by hemolytic streptococcus in local infections such as carbuncles sinusitis, infections of eye and ear, and particularly in urinary tract ailments the usefulness of sulfonamides has been very great. In many other infections such as gas bacillus complications trachoma, actinomyces and many other diseases, it has been of some help. The usefulness and limitations of the sulfonamides are today well recognized by the medical profession.

MENINGITIS

History tells that there were continuous epidemics of meningitis during the Napoleonic wars. The meningococcus, the germ which frequently causes epidemics of infections of the brain cover, can be transmitted from person to person. The germs are found in the throats and noses of persons who may themselves be healthy but who are, nonetheless, carriers of this germ. Under normal conditions of civilian life children under five, more often than adults, are victims of this disease. But in military barracks extensive epidemics are likely to occur. Such a devastating outbreak took place among the Canadian troops during World War I. Soon afterward similar epidemics of meningitis developed in various camps in this country. The disease stood fifth as a cause of death for servicemen in the United States Army. The mortality rate, was as high as 35 per cent.

In World War II the situation was completely changed. By the use of sulfonamides serious efforts were made to prevent epidemics of this disease. The mortality was kept very low, hardly reaching 5 per cent.

Four Army medical men were very successful in preventing the disease in their camp. Dr. M. Kuhn, Dr. C. Nelson

Dr H Feldman and Dr I Roland Kuhn conducted their instructive experiment in which several thousand servicemen acted as guinea pigs at a camp in rural Mississippi. Occupied only since August 1942 the camp housed fully 34 000 soldiers by March 1943. The following summer an epidemic of meningitis broke out seventy two cases were registered. It was of utmost importance that the spread of this dangerous disease be prevented. It was known of course that the illness is contracted mostly through the respiratory tract and that the germ of the infection is highly susceptible to the action of sulfa drugs. Why not try to prevent the infection by giving all the soldiers a small dose of sulfa drugs? Eight thousand men the entire personnel of a certain area, were selected for treatment. The remainder of the division ninety three hundred men barracked in two other areas served as the control for this experiment. Each of the servicemen in the first group received one tablet of sulfadiazine with every meal. The treatment lasted for three continuous days the whole course of treatment thus providing nine grams of the drug. The organization of the experiment was perfect. The drug was issued by the commanding officer of each unit as the men entered the mess hall. A noncommissioned officer made certain that the tablet was actually swallowed. Each man was advised to drink at least one and one-half litres of water every day in order to avoid injury to the kidneys. This routine was followed without a hitch. Both the treated and the untreated groups were kept under observation for several months. The first encouraging fact noted was that none of the treated men had the germ of meningitis in his nasal secretion while among the untreated group some of the apparently healthy soldiers were carriers of the germ. At the end of six months the experiment was proved a great success. While twenty three of the untreated soldiers fell vic

tum to the meningococcus, none of the treated men was affected

This indicates that, by giving small doses of the sulfa drugs the chances of contracting meningitis are greatly reduced. Naturally there would be no sense in, or necessity for, taking such measures unless there was the threat of an epidemic. Such preventative measures are justified only when a number of cases of meningitis appear in a limited locality.

When it became apparent that meningitis caused by meningococcus could be successfully treated with sulfonamides doctors breathed a sigh of relief. Here, at last, they had a remedy with which to fight a hitherto baffling disease. Since that time the majority of meningitis cases have been treated with various types of sulfa drugs. The results have been excellent. Until recently, no more than forty three persons out of every hundred stricken with this disease could survive; now ninety persons out of every hundred have an excellent chance to recover if they are given proper doses of sulfa drugs.

What compound is the most effective here? Dr. William W. Zeller and his associates of George Washington University Medical School, Washington, D. C., have tried various sulfa compounds on a number of patients affected with meningitis. They gave their patients sulfadiazine and sulfamerazine alone or a mixture of both substances. The mortality rate was 10.1 per cent in the patients treated with sulfadiazine or sulfamerazine alone, and 6.7 per cent in the patients treated with a combination of the two drugs. However, the doctors concluded this difference was not considered to be significant in view of the fact that the patients who received the combined therapy were treated at the end of an epidemic.¹⁶ Apparently the germ was less virulent and aggressive and more susceptible to the action of the

drug by that time Neither sulfathiazole nor sulfapyridine are recommended in meningeal infections

Even more striking are the results of the sulfa therapy in infections of the brain cover caused by hemolytic streptococci Twelve years ago before sulfonamides were known only two out of a hundred children stricken with this dreadful disease had even a meager chance to recover At present only a small percentage of children are likely to die not more than two or three out of a hundred treated with sulfa drugs *In the field of meningeal infections sulfonamides have rendered the greatest service to human beings*

GO\NORRHEA

There is always a great increase in venereal disease during wartime World War II was no exception An enormous number of servicemen were infected with gonococcus the agent of gonorrhea But fortunately sulfonamides were available and revealed exceptional efficiency in the treatment of this disease By the use of this drug gonorrheal infections among Army men were cured promptly before the men might become chronically infected

Drs Hyman Strauss and Isaak Grunstein of the Department of Hospitals City of New York tell of their experiences in using sulfa drugs on five hundred women infected with gonorrhea In some instances the women had chronic venereal diseases in others recent infections All were hospitalized at the Kingston Hospital in Brooklyn They were repeatedly examined and complete records of their case histories were kept In order to check which dose of sulfa drugs was more effective the patients were divided into three groups One group received three grams of sulfathiazole daily for ten days the second group was given four grams daily for ten days while the third group received four grams daily for seven days The significant fact was that the first group

showed less satisfactory results than the other two. While in the first group the rate of recovery was only 84.2 per cent, in the other two the rate of recovery was respectively 92.3 and 95 per cent. This investigation indicates that in the treatment of gonorrhea higher doses given for a shorter period of time may bring better results.

Not only are the sulfa drugs effective in fresh or chronic infections of gonorrhea, but they can bring recovery, or at least relief, in the arthritic complications of this disease. Gonorrheal arthritis is a most painful and stubborn affliction. The germs spread into the joints of the fingers, arms, and legs, in fact to almost all the joints of the body, and quickly transform a healthy man or woman into a complete invalid. Sometimes the progress of the infection is so rapid that within four weeks the person is unable to walk or even to move an arm or leg. Until sulfa drugs were discovered, there was no effective remedy. But in cases of gonorrheal arthritis, sulfonamides perform miracles of recovery. In one case, a man who had been confined to bed for several weeks with progressive gonorrheal arthritis was able, after sulfonamide therapy, to walk again and to return to his job. In the past ten years sulfa drugs have cured many hundreds of thousands of persons infected with gonococcus.

PNEUMONIA

In 1937, sulfa drugs were little known to the medical profession. Early reports about the outstanding antimicrobial properties of these drugs had just appeared in the medical press, and very few physicians prescribed them to their patients.

At this time an internationally known artist became gravely ill with pneumonia. He was very old indeed, and his heart was no longer in good condition. The examination revealed that his lungs had been invaded by the common offender in

was gained poliomyelitis is an inflammation chiefly of the anterior horns of the spinal cord. Although the incubation period may vary, it usually runs about ten to twelve days. The first symptoms may be very mild consisting of slight fever and pain in the head and neck. Intestinal symptoms may or may not be present. As the disease progresses the symptoms may take various courses. In some cases the intestinal symptoms dominate, while others show signs of cerebral irritation. Poliomyelitis cases may be divided into two general groups.

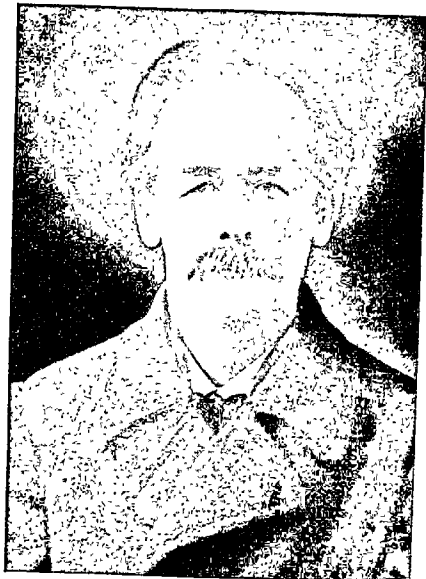
In the first, the persons affected never become paralyzed. These are called abortive cases of polio. In the second, bulbo-spinal group paralysis usually does occur.

Drs. S. Flexner and P. Lewis disclosed, as early as forty years ago, that the virus of polio can be transmitted to monkeys by injection. They found the virus not only in the spinal fluid of infected monkeys and men alike but in the blood and nasal cavities as well. Thus they established that the polio germ can be transmitted from person to person through nasal secretions. It was also discovered that the virus of polio in milk or water can penetrate the system of man through the intestinal tract. A person who has recovered from polio is immune to new infection. Flexner demonstrated that the blood of persons recovered from polio has some mild destructive properties against the virus of this disease. However, as yet no potent serum has been developed.

It is interesting to note that the blood of healthy persons has been found to contain antibodies of the polio virus. Some investigators including Flexner, are of the opinion that a large number of persons suffer a very mild form of polio from which they recover, without ever knowing they had the disease. They assume that there are probably hundreds of cases of the mildest form of polio to every actual case of grave infection. These hundreds of persons remain immune to the



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infection for the rest of their lives. This fact may explain the distressing frequency of polio among small children. They have no immunity whatsoever against polio. But there are also a goodly number of adults who may lack the protective element in their blood. In such cases, as with our late President, Franklin D. Roosevelt, grown men fall victims to this disease.

It is no wonder that the work of Dr. Saunders has attracted great attention, from the medical profession as well as from the public at large. The data concerning his discovery is not yet complete, and so far the clinical evidence has not been published.

Following its discovery by Dr. Saunders, darvisul was developed by Dr. M. E. Hultquist and Dr. Robert Parker at the Calco Division, American Cyanamid Company, Bound Brook, New Jersey. The new drug is a white powder which is almost insoluble in water. For this reason a sodium salt of the compound, more soluble and apparently nontoxic, was prepared. When injected into an animal it does not produce any harmful reactions; animals can stand very heavy doses without ill effects. Like sulfonamide, phenosulfazole does not directly destroy the virus of polio, but rather acts upon the cells of the body, and protects them from invasion and assault by the polio virus.

An extensive investigation has been conducted with the drug on mice and monkeys. For these experiments the strain of virus originally isolated at Columbia University by Dr. Claus Jungeblut, an expert in polio, and Dr. Saunders was selected. Mice infected with the polio virus, and soon afterward treated with the drug, were cured. Hence the drug was able to arrest the disease in its early stages. The mice that survived were immune to infection. But the most interesting point was that a single dose of the drug prevented infection in the animal. Similar results were obtained with monkeys.

They were protected from the ravages of poliomyelitis by a single dose of the drug given by mouth. They recovered from the infection in its early stages

Information about clinical investigations is still very sparse. The drug was tried in Texas during the recent epidemics of polio. Some of the tests were failures it was said. As Dr. Saunders stated: "We do not know what effect darvisul has on human poliomyelitis. Premature claims for a polio cure are not only unjustified but under present circumstances criminal."¹⁸

More recent clinical investigations indicated that darvisul is of little if any help in the treatment of human poliomyelitis.

TOXICITY OF SULFONAMIDES

When Paul Ehrlich had discussed with Dr. O. Kateret, his friend and associate, the magic drug for which he was searching, he repeatedly stressed that an ideal antimicrobial drug should be completely harmless to the human organism. He was equally concerned about the effectiveness of the drug and its toxicity. This postulate remains unchanged from the time of Ehrlich. It should be emphasized that low toxicity in a drug is a necessary prerequisite for any ideal or near ideal antibacterial substance. From a physiological viewpoint to kill the infection only to cause damage to the organism is no solution.

What constitutes low toxicity or harmlessness, in a drug? The first question to be posed is: how does the drug affect the white cells, the leucocytes, of the organism? It has been established that the natural defense forces of the organism are of prime importance in the fight against invading bacteria. These forces should not be weakened or impeded by the drug, the goal of which is to fight the infection.

How do the sulfonamides act on the white cells? Suppose

normal healthy rabbits are given injections of sulfanilamide the same dose (per kilogram of weight) as would be given to a man in case of an infection. The treatment should be continued for six or seven days, as is usual in sulfonamide therapy. Now, if the blood count of the rabbit is taken after he has received the full dose of the drug it may be noted that the leucocytes of the rabbit instead of being active and mobile, are passive and completely lacking in migratory activity. Gradually they degenerate and perish. This finding indicates that sulfonamide has a toxic effect upon the white cells. These cells when deprived of their vitality and migratory ability are useless in the fight against germs. However, several days after the treatment has been stopped the white cells of the rabbit will again be quite normal and mobile. This indicates that the depressive or toxic effect produced by the drug on white cells is of a temporary nature.

In the blood of a man suffering from some infection who has been given regular doses of sulfanilamide for seven to eight days there will be a sharp drop in the blood cells known as granulocytes—evidence that the bone marrow is somewhat depressed by the drug. The leucocytes when examined in culture as in the case of the rabbits will show the same picture of immobility. There may be some decrease in red blood cells and the blood phosphates will drop in the plasma.

If the doses of sulfanilamide given to the patient are larger, or the treatment is prolonged the condition of the blood may become menacing. For it has been shown again and again that the sulfonamides have a destructive (hemolytic) action upon the red blood cells. A pediatricist who treated forty-eight babies suffering from pneumonia with sulfapyridine reported that in thirty-two there was a substantial decrease in the number of red blood cells.¹⁹

Considerable clinical evidence has accumulated in recent

years on the toxic effect of sulfonamides upon the blood and blood forming organs. It was found that anemia (due to the hemolytic action of the drug) is quite a common result of this therapy. In some cases when the dosage was large and was administered for a considerable length of time dangerous forms of anemia developed which caused the death of the patients.

The blood disease known as agranulocytosis might occur when the drug is given for more than fifteen consecutive days. This condition is very grave and often results in death. However some of the sulfonamides seem to be less toxic than others. Sulfapyridine is the greatest offender in this respect while sulfadiazine is more harmless. However while mild anemia is quite frequent in sulfonamide therapy serious damage to the blood system is relatively rare so long as the patient is treated at a hospital and his blood is checked frequently.

Drs. H. F. Dowling and M. H. Lepper who investigated the effects of various sulfonamides on the blood of 1,489 treated patients found that acute hemolytic anemia occurred in 1.4 per cent with sulfapyridine, 0.3 per cent with sulfathiazole, and 0.2 per cent in the patients receiving sulfadiazine.

Soon after the sulfonamides received general recognition and the drugs were widely applied in medical practice alarming information began to accumulate regarding the harmful effect of these drugs on the kidneys. Many physicians stated that patients developed kidney ailments as the result of this therapy. In some cases a formation of calculi was found in the kidneys. In others blood appeared in the urine and hematuria was present. Drs. W. W. Zeller and his colleagues observed kidney injury in 8.1 per cent of the patients treated with sulfadiazine and in 11.5 per cent of those who received sulfamerazine. Other physicians gave similar or slightly varying figures stressing the fact that sulfanilamide seemed to be a less offending drug in this respect than sulfamerazine.

and sulfadiazine. In most cases the kidney ailment disappeared as soon as the treatment was arrested. However, experiments on animals suggested that injury to the kidney from very large doses of sulfonamide might be permanent, the damaged kidney might never recover completely from the injury inflicted by the drug.

As an example, if a healthy dog were given a large dose of sulfonamide, which would correspond to the dose given to a human with a grave infection, with the treatment being continued for ten to fifteen days, or even longer, at the end of the therapy the dog's kidneys would show some destructive changes. Some of the tubules of the kidneys might be blocked and numerous small lesions might be present in their wall. Small calculi might be found, or an acute inflammation of the kidney tissue. However, when the doses of the drug are not very large, and the length of the treatment is not prolonged unduly, injury to kidney may be insignificant or even entirely absent.

The general consensus is that persons who suffer from kidney ailments are particularly predisposed to such complications from sulfonamide therapy. Therefore, in order to prevent injury to the kidney, it is recommended that considerable amounts of water be drunk during the sulfonamide therapy.

Some of the patients receiving sulfonamide complain of dizziness and mental foggiess. Much discussion has been carried on in the medical profession about these symptoms. It has even been suggested that industrial employees who are treated with sulfonamides should not be permitted to work during the period of the treatment. Possibly some people, weakened by the infection, might so react to the therapy. But actually, in otherwise normal persons, the drug does not produce any bad effects as far as mental conditions are concerned.

Dr. Alison H. Price, in collaboration with John Pedulla,

Safety Examiner of the Pennsylvania State Police undertook a large-scale experiment to prove that there is no need for concern about the mental state or absence of coordination in the people receiving sulfa drugs. They took one hundred thirty-four healthy medical students and divided them into two groups. One group served as the control. This group of forty-four students received no drugs at all. The other group consisted of ninety students who were given sulfadiazine for over seventy hours to a total dose of nineteen grams.

Before and after the therapy steering and vigilance tests were made. The steering test was to prove the presence of eye-hand coordination. The students were obliged to steer the car and keep it aligned with a small dot located in the center of a movable road scene realistically set up before him. The vigilance test measured alertness and ability to perform several acts at one time. The student was obliged to shift gears and to apply the brake as in actual driving in addition to steering. A special apparatus which simulated actual working conditions was used to measure the coordination and reaction time in the human guinea pig. Eye-hand coordination and reaction times were automatically recorded by an electric clock. There is no significant difference between the controls and those subjects given sulfadiazine, was the conclusion of Dr. Price and Mr. Pedulla.

This test has only a limited significance. But it does prove that a healthy man may take sulfonamide for three days in large doses without experiencing dizziness and mental foggy-ness.

However, in many instances there is a general toxic reaction to sulfonamides. It might manifest itself in the form of a fever similar to serum fever (a reaction to antibacterial serum). It has been observed that sulfathiazole causes such a fever more often than the other compounds. On the other hand, vomiting and nausea are present more frequently

in sulfapyridine therapy. Skin rash of various types and manifestations is often observed during sulfonamide therapy. Here again, certain of the sulfa compounds produce stronger reactions, while others offend the organism less.

Which sulfa compound is least toxic to our organism? According to Dowling and Lepper's observations, sulfapyridine caused toxic reactions in 29.9 per cent of their patients; sulfathiazole produced toxic effects in 11.8 per cent, and sulfadiazine in 7.7 per cent of the treated patients. Other investigators give somewhat higher figures for sulfathiazole and lower figures for sulfapyridine. From these observations it appears that *sulfadiazine should be considered the least toxic drug so far among the sulfonamides*. Yet it, too, must still be considered relatively toxic.

What conclusions can be reached on the toxicity of sulfonamides for man? The recent work of L. Lichtenstein and L. Fox,²⁰ of A. J. French, and of R. H. More and his associates²¹ strongly indicates that some persons are oversensitive to all the sulfonamides. These drugs may produce toxic effects of such magnitude and severity that the health, or even the life, of the person may be endangered.

It is impossible to figure out the exact percentage of people who are oversensitive to these drugs. However, these persons may react even to relatively small doses, if given for any considerable length of time. For this reason, sulfonamides should be administered to patients only under the constant surveillance of the doctor. The blood and urine of the patient receiving sulfonamide therapy should be checked every day in order to observe what, if any, effect the drug produces on the organism. No doctor can foresee his patient's reaction to sulfonamide. He never knows beforehand whether or not the patient will be hypersensitive to these compounds. Even if the patient has tolerated sulfonamide therapy quite satisfactorily in the past, his reaction to a second or third course

of treatment might not be the same for he may have become sensitized to the drug, and may manifest violent toxic reactions

Therefore it must be emphasized that *sulfonamide drugs are of relatively high toxicity and should be administered to the patient with the utmost care. By no means are they ideal drugs, for they do produce toxic effects*

Sulfonamides are not the magic bullets of Dr Ehrlich's dreams. They are not even the ideal antimicrobial drugs that every physician would like to have at his disposal for they are relatively of considerable toxicity. And yet so far as antimicrobial activity is concerned they are truly remarkable. They are very active against the most common pathogenic bacteria which so often cause dangerous and even fatal infections.

When properly administered the toxic reactions from the use of sulfonamides can be greatly reduced or even prevented. They have undoubtedly saved hundreds of thousands of human beings who would otherwise have been unable to survive their grave infections.

Through the discovery of sulfonamides chemotherapy has regained its status in the medical world. The days of faith in magic drugs have returned with the sulfonamides. It is true that these compounds were not discovered or even suggested by Paul Ehrlich. But in all fairness one must admit that sulfonamide is the child and creation of his powerful mind. The idea of a magic bullet prepared from the azo-dyes found its realization in the magic drugs still being prepared from the same azo compounds. The entire approach in sulfa drugs is the logical continuation of the side chain ideas which Ehrlich treasured from his student days.

4

When Dog Eats Dog

THERE IS A short rather narrow street in Paris almost hidden by thick foliage, and not unlike a number of other Parisian streets. But something about it sets it apart. Taxis do not spoil its serene charm. People walking along it seem a race apart. In ill fitting badly pressed clothes they stroll here slowly, with the transported look of those who hardly belong to the world of reality.

Officially this street is listed as the Rue Dutot but it is more popularly known as Rue des Sciences because the laboratories of the Pasteur Institute are located there.

This street has long been associated with impassioned stubborn and unyielding battles of ideas. Some of these struggles have been carried on for years and even for decades. The very air of this street seems to be charged with ideas some dead and some alive some frustrated and already disproved, others established triumphantly.

Along this street Louis Pasteur often strolled, leaning on his stick, deep in concentration, always looking straight ahead of him as though peering into the future. No longer a young man, his fame was at last acknowledged even by his foes. His entire life and all his interests had been devoted to one pur

pose science For him science was neither an abstraction nor an escape from the realities of life It was a never-ceasing fight on those enemies of mankind, the disease-producing germs This was his personal war, one he waged throughout his lifetime with that militant perseverance which sometimes assures success When his life ended in 1895, the fight was no more his alone For he bequeathed his knowledge and his purpose to his colleagues and disciples and urged them to continue the war with equal persistence in the great institution that bears his name

Do not fear to defend new ideas even the most revolutionary Your own faith is what counts most But have courage also to admit an error as soon as you have proved to yourself that your idea is wrong Science is the graveyard of ideas But some ideas that seem dead and buried may at one time or another rise up to life again more vital than ever

In the laboratories of the Pasteur Institute the spirit of this great scientist is still alive His indomitable enthusiasm and will to win continue to be an inspiration to the generations following him

One of the rooms on the third floor houses a curious museum, an exhibit which graphically presents the story of the fight against the disease-causing germs On these shelves stand hundreds of test tubes labeled with the names of the microscopic foes already vanquished or at the point of surrender Typhoid, the plague, cholera, diphtheria tetanus and many others—these are the scourges of mankind that are arrayed side by side in meek subjection, as it were, in this chamber.

Charts explain to the layman the extraordinary power of these terrors Dr Danish, Pasteur's colleague calculated that, with proper food supply, and nothing else hindering them the cholera germs could multiply so rapidly that within two weeks they would completely cover the surface of the globe

As a matter of fact, so great is the number of descendants a single cholera germ can produce in one day that it would be difficult to express it in figures; one would have to employ a standard of weight. For under imaginary optimal conditions this number would reach a thousand tons in weight *at the end of the first day*.

The same museum contains a shelf of exhibits of bacteria of another sort. These are the friends of man, the organisms that help man to combat the disease-causing germs. Whereas Pasteur, Roux, and the other workers at the Pasteur Institute centered their attention primarily on our enemies, the disease-producing bacteria, Metchnikoff and his associates became interested in the benefits mankind might derive from the friendly bacteria.

With Metchnikoff this study was almost an obsession. No other quest among his countless researches appealed to him or intrigued him quite as much as the struggle which he observed among the various species of microbes. Even if he had to interrupt his current work, he could hold forth on this subject at any time with animation and excitement.

For us youngsters in science, who had come to the Pasteur Institute to work in the laboratory of the famous Metchnikoff and sit at the feet of a man who was a close collaborator and friend of the great Pasteur himself, this pursuit of his favorite topic was often annoying. Metchnikoff probed deeply into the underworld of life, the world of microorganisms, which he visualized as existing in a state of constant turmoil, in a cruel struggle for existence. He realized that the human body furnished a particularly favorable terrain for these microscopic forces. But for the unseen gigantic war amidst these tiny invaders, the human body would not have a chance. Mankind would have perished long ago, as soon as the species Man began to live a tribal life.

"Observe that fantastic power of proliferation which these

small bugs possess," Metchnikoff pointed out, showing us plates on which, in one day, the germs had developed from a small insignificant colony to an enormous, indeed ominous, mass "Just give them free rein and favorable conditions, protect them from other germs, and they would cover the whole globe destroying mankind in little time. Fortunately for us at every step they meet enemies that are ever ready to devour and to destroy them

Man is ever the bearer ' the scientist continued, "of every kind of disease producing germ You are none the worse because they are in a state of 'depression' Yet here they are, alive if not active, these potentially mortal enemies of yours.

"Now you are young and healthy," he gestured toward a student, ' but I assure you that I could find in your mouth in your sputum, and in your intestines more than a couple of dozen of these dangerous disease producing germs "

He then demonstrated his statement An apparently healthy individual would be found to be the bearer of the tubercle bacillus, or the influenza germ or *Bacillus paratyphosus*, plus a number of other pathogenic (disease producing) germs¹ "Now, I ask you, why are these germs that we have found in this young man in this latent or passive state? Why are they content to conceal themselves so timidly in the corners of his lungs, his colon, his mouth? Is it because of natural resistance? ' Metchnikoff speculated 'Or because of acquired immunity? Surely that is only a partial answer to our question. There must be another factor which retards the dangerously swift development of these germs in the human organism, and that cause must in some way be connected with the presence of other harmless, even friendly bacteria, which exercise a destructive and restrictive power over our enemies These friendly bacteria are usually native to our bodies. They are aborigines, we might say, who use some chemical weapon

against the invaders, the nature of which we are unfortunately ignorant.²

Thus, while Ehrlich, Wassermann, and other leading scientists explored the field of synthetic drugs in search of germ killers, Metchnikoff was approaching the same problem from an entirely different angle. He was fairly obsessed by the idea that there existed—that there should exist, a natural germ-killer among the substances produced by the friendly microbes. 'There must be some microorganism which possesses a perfect chemical weapon to kill pathogenic germs.'

But how to find it? Metchnikoff and his associates made several attempts to solve this problem. Their efforts bore little fruit. At that time the development of biochemical methods was still in a primitive—or let us say, an embryonic state. Yet some of their efforts are as colorful as any adventure story, and worth remembering.

The problem of tuberculosis has always occupied a hal-
lowed niche in the research laboratories of the Pasteur Insti-
tute. One might say it was one of the specialties. Albert Cal-
mette, Alexander Besredka, and many others devoted their
time and energy almost exclusively to it.

The tubercle bacillus (otherwise called *Mycobacterium tu-
berculosis*) is extremely resistant to unfavorable conditions
more so than any other disease-producing germ. In water and
in sewage, tubercle bacilli can remain viable for weeks. In
dried sputum they frequently retain their vitality for several
weeks, and even months. In fact, virulent tubercle bacilli have
been found in dried sputum as long as ten months later.³
Cold has little effect upon these germs. When dry, some of
them remain alive even after being heated at 100°C (the
temperature of boiling water) for twenty minutes. The re-
sistance of this bacillus to chemical disinfectants is likewise
very strong. In sputum it will resist the action of a 3 per cent
solution of sulfuric acid.⁴

Naturally these facts make this germ much more dangerous and difficult to treat. But *why* is this germ so resistant? The answer is simple. Tubercle bacillus has a thin waxlike coat—a capsule. This waxy capsule a very ingenious and practical protection provided by nature guards the microorganism from cold and heat as well as against chemical agents. The question is how can the germ be attacked?

Metchnikoff had his own ideas. One day he called in one of his assistants a brilliant biologist Dr Serge Metalnikoff later to become a leading member of the Pasteur Institute.

We know Metchnikoff told him that the tubercle bacillus has a waxy covering. When and if this waxy capsule is destroyed the germ perishes. Therefore it seems to me we must find an agent that will dissolve this capsule, an agent that will liquefy the wax. Your problem then is to find a microorganism which has the unusual ability to digest this wax.

The problem assigned to the young Russian scientist was not a simple one. No such microorganism had ever been found or described. For some time Metalnikoff was at a loss. Where could he direct his search for this hypothetical microorganism which probably existed only in the imagination of his chief? Where in nature is there a bug which can digest wax? he reasoned.

One day a thought struck him: wax—honey—bees! The honeycomb is made of wax. There may lie the answer to my problem, thought Metalnikoff. He plunged zealously into an investigation of the life and procedure in the beehive. Are bees hosts to any parasites which make beehives their home? Such a parasite might be able to eat through wax.

The young scientist's inspired search was soon rewarded by a measure of success. He discovered that the only insect in the world other than the bee that subsists on honey is the caterpillar of a small gray moth which as a rule flies only at night. The Latin name of this moth is *Galleria mellonella* the last

word evidencing its love for sweets. Yet the name is somewhat misleading, for the butterfly itself does not care for honey. However, its caterpillar is a true honey-devouring larva, for it eats nothing but honey.⁵ It robs the hard-working bees by invading the hives and by boring holes in the honeycomb to get at the honey. This arrogant little creature sometimes causes severe damage to the honey industry.

As soon as Metalnikoff had located the wax-eater, he subjected it to a most thorough investigation. For more than three years he studied the habits, the physiology and the chemistry of this unusual caterpillar. He examined it minutely and soon found that both the digestive system and the serum of this creature possessed an exceptional ability to digest wax.⁶

That was the first step. Now the scientist proceeded to the more difficult portion of his assignment. Would the juices of this honey-eating caterpillar destroy the tubercle bacillus? His experiment was simple but ingenious. He took a live culture of tubercle bacilli and introduced it into a caterpillar's abdomen. Hour after hour he watched these germs under the microscope. What he saw was fascinating. The waxy capsule of this stubborn foe of mankind, so resistant to most chemical substances, was gradually falling apart, melting away like snow under a warm sun. Eventually, disrobed of their protective capsules, the dangerous bacilli perished. The wax-digesting juices killed them all.

Again and again Metalnikoff repeated his experiment. He was finally convinced that the serum of his caterpillar unquestionably destroyed the bacillus. If such was the case, could one expect serum to be as effective in a test tube as in the body of the caterpillar? He began a new series of painstaking, time-consuming tests. He extracted the juices from a dozen caterpillars into a small tube. To this liquid he added a large amount of living tubercle culture in a special medium. Again,

more anxious than ever, he watched the action of the extract on the germs. This was the real test. Would the juices work again? Or would the germs remain unaffected. Victory again! The waxy capsules of the germs were destroyed in the tube as they had been liquefied in the body of the caterpillar. With this destruction came the quick end of the bacilli themselves.

The young Russian was not the only one thrilled by this discovery. The entire Institute was stirred. The young scientist's chief, Metchnikoff, was especially pleased. For was this not more proof for his theory, if indeed more proof was needed?

Yet Metchnikoff's investigations were far from completed. The most important part lay ahead. The idea was sound, but was it practicable? Could a similar method be applied to the treatment of human tuberculosis? First he had to prove that animals infected with tubercle bacilli could be cured.

Here he met his first setback. When he began to treat a tuberculous guinea pig, he found that to achieve success he needed enormous numbers of these honey eating caterpillars. Not hundreds, but thousands of these small creatures would be needed to supply him with the precious serum. Yet he had in his possession only a limited number of caterpillars. In the end he was able to treat a few guinea pigs. To his great satisfaction he cured them, but there his work had to end. The practical difficulties he could not overcome. To make a test on human beings, he would have needed *tens of thousands* of these caterpillars, and special technical facilities for extracting and preparing the serum.

So far as is known, this method has never been tried out on a human being. Frankly, we do not know if it would have the same effect as on the laboratory animal. The idea seems to be sound and to have possibilities, yet its practical value today seems doubtful.

This work, which made quite a stir in the scientific world

three decades ago, is now more or less forgotten. All that is left of this unsuccessful attempt, which Metchnikoff instigated to kill one germ with the help of another, is a monograph by Dr. Serge Metchnikoff which reports the results of his investigation. Published by the Pasteur Institute it reposes undisturbed on the shelves of medical libraries, a symbol of the power of human imagination and ingenuity in research.

Bacteria fight each other for their lives. This struggle goes on uninterruptedly. But how, with what implements, do these infinitesimal organisms carry on their wars? They have neither teeth nor claws, nor any of the tools with which more highly developed animals wield destruction. Their weapon must be chemical, the nature of which is still very much a mystery.

This was the problem that intrigued Metchnikoff. If we could only penetrate the mystery of interbacterial conflict to decipher the essence of chemical warfare. His attention was naturally directed to the human body, as the area in which the outcome of such struggles is of greatest consequence. The large intestine, the colon with its various and numerous bacterial inhabitants, became the subject for his intensive investigations.

Metchnikoff was struck by the fact that a healthy person with normal digestion has predominantly acidophilic flora. This means that a large number of the bacterial inhabitants of the colon belong to the group of bacteria which not only prefer a slightly acid environment, but are also able to produce an acid. A prominent member of the group is *Lactobacillus acidophilus*, which is indigenous to the human body. In fact, newborn infants are free of intestinal bacteria, except for those of the *acidophilus* group. When, however, a person is affected with some sort of intestinal infection, such as dysentery or typhus, or even when he merely suffers from chronic constipation, this 'friendly organism' as Metchnikoff called

it, sharply decreases in number or disappears altogether

Everything in the world of living matter, and in the organization of the body mechanism, has its purpose and its reason. There must have been some good reason, Metchnikoff pondered for nature to have endowed the human colon with this friendly microbe. What is particularly distinctive about it? Lactobacilli, by attacking and breaking down various carbohydrates, can produce lactic acid in considerable quantities. Lactic acid is not only a mild antiseptic, but is in itself a nutritive substance. It does not destroy every disease producing germ but only those which most often cause intestinal infection and which cannot thrive in acid surroundings such as the germs of typhus fever or of dysentery. Unfortunately, lactic acid is not effective when present in too small a quantity or in too low a concentration. A perfectly healthy human colon, according to Metchnikoff, has a continuous supply of this acid due to rich lactobacillic flora. This substance protects the colon against infection. A diseased colon however, obtains this antiseptic substance in amounts too insignificant to be effective.

If only men were able to keep their colonic flora predominantly acid forming. Metchnikoff averred, many intestinal maladies could be prevented or cured. This was the premise of his famous theory which at one time received much attention from both the medical profession and the public. Cultivate and encourage the friendly microbes capable of destroying the disease producing germs in your colon and thus you will keep your good health and defer the afflictions of old age.

That was the essence of Metchnikoff's theory. In the main it was sound and still remains so. There is no doubt in the minds of contemporary scientists that the lactobacillus is a classic example of a bacterium which is friendly to the human organism, since it produces a mildly antiseptic substance which is completely harmless and even beneficial to human

health But by some irony nature has endowed lactobacillus with a fundamental weakness It is a very delicate microorganism, easily destroyed by the unfavorable conditions which only too often exist in the human colon Lactobacillus could be described as a not very strong fighter, easily knocked out by other bacteria

To keep the colon normal and to replenish the supply of lactobacilli in the ailing human organism Metchnikoff advocated the implantation of these friendly bacteria in the intestine His advice was to ingest cultures of the lactobacilli by drinking yoghurt, a fermented milk beverage But he did not foresee that such implantations are rarely successful These bacteria, being peculiarly sensitive to adverse conditions might never reach the colon their ultimate destination Most of them perish during the long journey through the small intestine This fact presented such a serious obstacle that Metchnikoff's theory finally came to be regarded by the medical profession as practically groundless⁸

Many years later, Dr L F Rettger and his associates at Yale University actually proved that not less than a hundred billion acidophilus bacilli would have to be consumed daily to obtain a therapeutic effect! If however, such large quantities of the acidophilus could actually be given to the patient, the hostile, disease-producing germs would disappear from his colon, and his health might be restored Thus, while Metchnikoff's theory was fully confirmed in principle, its practical application, at least in the manner he suggested was impossible⁹

Once, years ago I was present at an informal tea in Metchnikoff's laboratory at the Pasteur Institute Afternoon tea was more or less a custom among the dozen or so scientists who crowded into the small laboratory and discussed the latest developments in the world of medical science over cups of strong Russian tea At times the debates on some fundamental

problems of research would become heated. Spirited arguments ensued, not always conducted in the politest manner.

On that particular day someone brought up the question of the paradoxical discovery made by Dr. R. Emmerich.¹⁰ He and Dr. O. Low had found that the bacillus of green pus itself a disease-producing germ, was in its turn a germ-killer. According to these scientists, this bacterium produces a substance which destroys the microbe of cholera (*Vibrio cholerae*) as well as the anthrax bacillus. The latter two diseases had always attracted the attention of the Pasteur workers. The anthrax germ study was something of a tradition. For it was while working on anthrax that Pasteur had started on his fabulous career. It was he who invented the method of preventing this acute and mortal infection among sheep and cattle by immunization with attenuated virus. He had saved hundreds of thousands of cattle and sheep and to this day his ingenious method remains the only one used.

Cholera, on the other hand, was of immediate interest to the workers associated with Metchnikoff, for an acute epidemic of that disease was devastating Russia, with many thousands of deaths reported daily. The majority of Metchnikoff's assistants were of course, Russians, obviously any remedy against this violent infectious disease was of utmost importance to them. Metchnikoff himself was stirred by the discovery made by Drs. Emmerich and Low. For here at last was a concrete observation that one germ did prey on another by means of a chemical weapon. (Although as early as 1877 Pasteur himself had observed some bacteria inhibit the growth of the anthrax germ.)

Dr. Emmerich's discovery was exciting to say the least. The green pus bacterium, frequently present on a healthy man's skin as well as in water, is sometimes responsible for serious infections. In wounds the presence of this green-blue-pus microbe, called *Bacillus pyocyaneus* (otherwise *Pseudomonas*

aeruginosa), greatly delays the healing process for it infects and forms a pus of a greenish or bluish color. During World War I this microbe was often the cause of chronic wound infections that were the bane of Army doctors. Yet in spite of its pathogenicity, *B. pyocyaneus* produces substances which have a detrimental effect on other disease producing bacteria.

Metchnikoff enthusiastically urged his disciples to study the chemical nature of the mysterious antibacterial substance produced by the microbe. By then he was already ill advanced in years and unable to do much himself. At his suggestion work on *B. pyocyaneus* was actually initiated at the Pasteur Institute, but Metchnikoff died long before the early results of the investigation were published.

When Emmerich first discovered the antibacterial properties of the green pus microbe he attributed them to a certain substance produced by this bacterium which he called pyocyanase. He thought that this substance was a ferment, an enzyme. Later investigations have refuted this assumption.

Actually, the picture of the antibacterial production of *B. pyocyaneus* is much more complex than that presented by Emmerich. This peculiar bacterium appears to be a manufacturing plant for several chemicals. We now know that three different substances are produced all of which impede the growth and affect the life of other germs.¹¹

The color of the pus produced by *B. pyocyaneus* when it invades the flesh varies from light green to almost dark blue. Both pigments are produced by the germ. One of these, a fluorescent green is common to many bacteria and does not deserve any special attention. But the blue pigment, which is called pyocyanin is a highly interesting substance. First described in 1921, it was investigated by Dr. A. Goris and Dr. A. Liot at the Pasteur Institute.¹² The pigment (when obtained

* "B" for bacillus.

pure it takes the form of long needle-like crystals) possesses truly remarkable properties. Not only is it antagonistic to other bacteria, inhibiting their growth, but in a weak solution it is almost harmless to animal tissue.¹³ In fact, it stimulates the respiration of living cells. In its presence the consumption of oxygen is greatly increased, and even insignificantly small amounts may augment oxygen consumption over twenty times.¹⁴ Paradoxically, pyocyanin while inhibiting the growth of bacteria, stimulates the multiplication of body cells. Thus the blue pigment affects the cells of the organism and the bacteria in different ways. Obviously—and this has already been proven experimentally—if pyocyanin is administered to an animal affected with cancer, the pigment may stimulate the cancerous growth. An animal thus treated will succumb to the disease much sooner than an animal that has not been so treated.

Besides the blue pigment the extraordinary organism manufactures two other substances with unique properties. The one, commonly known as pyocyanase is capable of fighting what was once the dread of childhood—the germ of diphtheria. When this remarkable property became known, many attempts were made to treat diphtheria with pyocyanase. Medical men applied pyocyanase solution directly to the infected throats of diphtheria patients, and often noted improvement in their conditions.

The other, a colorless substance which has no name has the specific ability to destroy the germ of cholera. When it was discovered it created a furor in Metchnikoff's laboratory. There was no doubt as to its effectiveness. It was shown to be capable of annihilating the cholera germ in the test tube as well as in vivo, that is when tested on animals. There was even a mild attempt to treat a cholera case with it. Yet neither of the two substances became popular with medical men.

A few years later a new report was published by the workers

of the Institute Dr Leo Rosenthal had discovered that the bacteria from the group of *Tyrothrix* (sporulating aerobic bacilli), could destroy the germs of cholera and typhoid fever¹⁵ He tested a number of *Tyrothrix*, among them *T. scaber* and *T. lenius*¹⁶ In the test tube it took about five days to achieve complete destruction of the *Vibrio cholerae* The bacteria with such peculiar germ killing properties were isolated by Dr Rosenthal from a cheese

In the course of his investigations, Rosenthal isolated from the soil another bacterium, which he identified as *Bacillus mesentericus* This bacterium could also destroy disease-producing germs but not as effectively as *Tyrothrix scaber*

What became of this new discovery? It was forgotten as soon as the results were published The work aroused the interest neither of the medical profession nor the bacteriologists Not yet did the notion of fighting one germ with another appeal to the scientific world at large

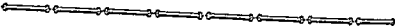
Yet though unrecognized and suppressed, the idea persisted As time went on, a brief isolated report would come to light here or there, about the germ killing properties of certain bacteria and molds However, such reports never reached the front pages of any medical journals No one paid serious attention to this field of investigation except a few stubborn souls who labored in obscurity, lacking, more often than not, the proper facilities for such expensive research

Not a single chemical or pharmaceutical company ever donated a penny to these investigations. Only with the dramatic discovery of penicillin or rather with the recognition of its practical potentialities as a drug did the idea in which Metchnikoff had believed so passionately receive new impetus and deserved recognition from the scientific world



5

Discovery of Penicillin



ON A COLD and foggy day in February 1910 a patient was lying on a cot in Radcliffe Hospital at Oxford University. He was a typical British policeman in the prime of life, big and husky of frame, forty three years old. On this particular day his condition was very grave indeed. The doctors who had examined him held little hope of saving his life. He was half-conscious and was running a fever of 105° . On his face and neck grotesque swellings forced his eyelids shut. Now and then painful coughing racked his body and drained it of its strength. To any medical man the diagnosis was plain enough: the dread septicemia. The hostile pus-forming bacterium called *Staphylococcus aureus* had invaded his blood stream and lungs. The man was dying of blood infection.

His illness had begun undramatically, as blood infections often do. Several weeks earlier, during a hurried morning shave, he had slightly cut the corner of his mouth. He thought nothing of it. Who would? But a few days later a small sore developed over the cut. A few times the scab fell off only to recur as a bigger and nastier sore covering more and more skin surface. One day his entire face seemed to become in

flamed Suddenly, the first symptoms of blood infection appeared

To the doctors the man presented a problem as old as time itself What could save him? Sulfa drugs? He had already been so treated without the slightest sign of relief Now, as a last resort, it was decided to treat him with an entirely new drug, a remedy that had never before been tested on any human being True enough, penicillin, as the new drug was called, had saved experimental animals, it had protected mice and rats against infection caused by the same germ, and when injected in small doses, was harmless to a healthy person

But who could say that the large dose required might not prove harmful when given to an emaciated and desperately ailing man? It might prove dangerously toxic Taking all these points into consideration, it was finally decided that, since no other remedy had availed this unknown drug should be given a trial

Dr H W Florey and Dr E Chain of Oxford University took charge of the experiment On hand they had but the limited quantity of penicillin which they had prepared themselves Their hearts filled both with doubt and hope they set out to treat their first case with the new remedy

Every two or three hours a relatively large dose of penicillin was injected directly into the patient's blood stream On the first day there was not much change, but at least the patient grew no worse On the second day, however, a slight improvement was noted The temperature went down slightly, the facial swelling diminished After five days of treatment the patient seemed to be on his way to recovery or, at least, so everyone thought Then the doctors discovered that they had exhausted their supply of the drug

Hurriedly they started to prepare more penicillin But for a few days the injections had to be halted The illness renewed its assault The temperature mounted, before they had pre

pared enough of the drug to resume the treatments, the patient died from an acute infection of the lungs. The doctors had lost the first round of the fight.

As far as the new drug was concerned, however, they were not altogether discouraged. Their first experiment had taught them a good deal. Now they were sure that penicillin was not harmful to human beings. It could be given even to those who were exhausted by illness without producing any bad effects. They had also learned that penicillin must be continuously present in the blood and system of the patient, until the infection is completely destroyed. This meant that the drug must be given to the patient without interruption hour after hour, for several days.

Armed with this knowledge they tried the drug on another patient. Their second case was a young boy of fifteen who had a badly inflamed hip joint. Now the battle was to be waged against the aggressive and dangerous blood-destroying streptococcus. The lad was at first treated with sulfa drugs, but without improvement. The germs had already invaded his bloodstream and his condition grew worse and worse. Every hope of saving him had been given up. Only then did the two doctors decide to treat him with penicillin.

Again their supply of the precious drug was very small. But luckily they had collected the urine of their first patient. From this source they recovered penicillin they could use on the second patient.

Day and night every two hours a penicillin solution was injected into the boy's blood stream. The two doctors watched his temperature slowly go down, the swelling reduce and new life return. Now the boy could even open his eyes. A short while later he was able to swallow a few spoonfuls of soup. Still they wondered whether their young patient would survive or succumb. Could penicillin destroy all the germs? Was it capable only of arresting the infection, not of curing

it completely? The doctors continued the treatment, now harassed by doubts, now hoping for a miracle. And a miracle it was. For they were rewarded by the complete cure of their patient. They frankly admitted that they had never expected such success until now there had been no remedy for this type of blood infection, and sulfa drugs had failed in this particular case.

Strangest of all was the fact that this curative drug had been extracted from a mold, a close relative of some of the common fungi that cause cheese to become moldy, leather to turn green, and wood to rot.

The development of penicillin began accidentally. In 1922, in the small, old fashioned laboratory of St. Mary's Hospital at the University of London, Dr. Alexander Fleming, the bacteriologist, found that human tears and saliva contain a substance capable of destroying germs. What was the nature and chemical structure of this mysterious material? This complicated question was to remain unanswered for two decades.¹ Dr. Fleming's laboratory had no facilities for chemical research of this kind, all he could establish was the fact that an unknown, strongly antibacterial substance was present in the human organism and was secreted in tears and saliva. He named this substance lysozyme and, unable to pursue this research further, left it at that point, after publishing a report.

Fleming's discovery attracted the attention of many scientists, including Dr. Jules Bordet, the famous bacteriologist and Director of the Pasteur Institute in Brussels. By a strange coincidence these two men are much alike. Both are short, retiring, and very gentle. Both are intensely concerned with scientific problems, and inclined more often than not to disregard the practical value of their discoveries. Bordet, formerly a student of Metchnikoff's, became very much interested in this natural antibacterial substance. He immediately

began to investigate lysozyme. Soon he confirmed Fleming's results and, moreover, found that the same substance was present in the white of egg.

In the meantime, two young scientists working in Bordet's laboratory made a startling discovery by pure chance, strange as it now seems, their findings did not impress Bordet much at the time. André Gratia and Sara Dath were working on the pus-producing *Staphylococcus aureus*. As so often happens, some of the plates filled with agar cultures had been contaminated by other bacteria or molds. One day, on one of the plates they found a strange, whitish mold around which the germ colonies were dying. It seemed as though the mold had a *destructive* effect on the pus-forming staphylococcus. Their observation was duly reported to Bordet. The workers were advised to follow up the new lead.

They soon identified the mold as *Streptothrix*, one of a group of so-called mold bacteria. This fungus killed the pus-forming germs easily, indeed it seemed to thrive on their destruction. For the mold on a clean agar plate grew slowly whereas it spread rapidly over the surface covered with the bodies of its victims.

Their attention riveted to this new phenomenon the scientists examined the plates every day, paying particular attention to the mold which was destroying the germs. Once, in an ampoule containing a vaccine, they found another mold, belonging to the family *Penicillium*, which seemed to have much the same effect. They neither attempted to describe it, nor perpetuated a culture. They were content merely to record their observations in two brief reports, which attracted no attention.² Today their names have almost been forgotten although they actually noted the existence of a mold germ killer a few years before the discovery of penicillin.

Exactly four years later Dr Fleming observed a similar phenomenon. After his discovery of lysozyme, his interest and attention had been concentrated on improving the technique of obtaining pure cultures of various germs. To find a method which would permit a quick diagnosis of the infection was the subject which preoccupied him. He experimented constantly with new and old chemicals and dyes to achieve a differential culture of a microbe and also studied cultures of the pus-forming germ. In the laboratory a number of culture plates were set aside for a few days, exposed to the air. The summer of that year was uncommonly cool and damp, and molds which prefer low temperatures and humidity, contaminated many of these plates. It is necessary to mention here that there is nothing unusual about the presence of molds on agar plates, mold spores are carried everywhere by air currents and grow wherever they can find food. However, on one of these plates the mold was acting peculiarly, destroying the colonies of bacteria. All around the mold the area was clear—the germ would not grow in its vicinity. Yet at some distance from the mold the germs were unaffected by its destructive power.

When Dr Fleming glanced over the plates he was struck by the strange phenomenon. Happily for mankind, the plates were examined by the doctor himself, else they might have been laid aside for washing and perhaps penicillin might never have been discovered. Dr Fleming immediately perpetuated his discovery by preparing a new culture of this mold. From this original culture are descended most of the molds used in the United States today for the production of penicillin by the surface method.

"When I saw the bacteria fading away, Fleming said later, 'I had no suspicion that I had a clue to the most powerful therapeutic substance yet found to defeat bacterial in

fections in the human body. But the appearance of that culture plate was such that I thought it should not be neglected."

What does this mold look like?

It looks somewhat like a brush. Dr. Fleming remarked when examining the mold under the microscope.

Much later Fleming admitted. At that time my knowledge of molds like that of most bacteriologists was rather less than elementary. But I could make out that it was a variety of *Penicillium* so the cultures made from the mold were named penicillin.

The molds captured by Fleming were similar to but not identical with the one studied by the Belgian scientists. They were distinguished by the peculiar structure of their sporophores. The fertile hyphae branched toward their ends terminating in a radiating cluster of flask-shaped cells. The whole spore head under low power magnification looked somewhat like a tassel or brush and this is the reason for the name *Penicillium*, derived from the Latin *penicillus*, a brush.

The mold grew rapidly a white fluffy mass the center eventually turning a dark green. Old cultures darkened to a greenish black. The mold produced a bright yellow substance which penetrated the agar in which it was grown. If however, the mold was grown in broth it changed in a few days to a dark-green mass.

As soon as Dr. Fleming had at his disposal a sufficient number of molds he was able to verify his initial observations of its destructive power with regard to staphylococci and other bacteria. He implanted the mold on plates filled with different pathogenic bacteria and studied the effects of the implantations. Soon it was apparent that these effects were in some instances very pronounced. On many plates the area immediately around the mold was completely free of growing germs. A particularly graphic lesson was visible on the plates of staphylococci or streptococci. Only certain types of

bacteria fell prey to this mold, while others continued to flourish in its presence. The next question, of course, was 'Is there a pattern of behavior which the germs follow? Which are sensitive to penicillin and which are resistant to it?' Dr Fleming soon found that the germs belonging to the Gram positive group almost exclusively were the ones affected by the mold. Staphylococcus, streptococcus, pneumococcus, among others, comprise this group, while the typhoid and dysentery groups and the influenza bacilli are not affected.

His next step was to apply penicillin's selective characteristic to bacteriological technique. If penicillin killed some germs and left others alive, he reasoned, here was an excellent agent for use in culture plates. He first prepared a crude extract of the mold, adding it to blood agar. Thus he had a medium in which staphylococcus, streptococcus and *B. diphtheriae* would not grow, but in which the germ of influenza would flourish. This ingenious technique allowed immediate identification of a germ taken from the mouth or throat of a case to be diagnosed. He found that the amount of penicillin required to prevent the growth of penicillin sensitive germs was insignificant. A dilution of one part to one thousand was effective.

Having satisfied himself that his method of cultivation was superior to all other techniques, Fleming decided to conduct an investigation on the presence of the influenza germs in the throats and mouths of healthy persons. Since the discovery of the causative agent of influenza, *Hemophilus influenzae* by the German scientist Richard F. Pfeiffer fifty years before, little progress in the prevention and cure of the disease had been made. Few people escape infection during the course of their lives, even when there is no epidemic such as the 'plague' of 1918-19. The only apparent explanation is that the germ is always present in the body, in a dormant state. Fleming set out to test this hypothesis.

It is a fact that as long as the individual remains healthy his resistance to infection is strong. But let him be exposed to inclement weather or be exhausted by overwork or malnutrition and the body cannot stop the germ from becoming active and aggressive from infesting not only the nasal cavity but also the lungs and the blood stream. Fleming strove to prove that the germs do not enter the body at this time but merely wake up and live.

With the help of Dr. I. H. Maclean he subjected one hundred healthy persons to bacteriological examination using the penicillin medium. In the first group were ten medical students, ten dental students and ten nurses all from the University of London. These people were all in perfect health with no indications of sinus trouble or any other nose or throat infection. In every instance the influenza germ was shown to be present when nasal or pharyngeal smears were placed on a penicillin treated culture plate. Fleming's theory was borne out.

Paradoxically the influenza germ is not highly resistant. It belongs rather to a group known to be sensitive to many unfavorable factors such as light, water and mild disinfectants. Moreover it cannot grow in the presence of other germs being checked by other bacteria. Nevertheless it resists the onslaught of penicillin to which most aggressive germs such as the cocci are highly susceptible. The most effective germ killer ever found is powerless to destroy the weakest of germs.³

Penicillin was discovered and described by Fleming in September 1928. At that time his work received very little attention. Scientists though amazed and intrigued by this discovery had little confidence in its practical potentialities. The idea was too revolutionary to appeal to the conservative minds of medical men.

Almost immediately after Fleming had published his re-

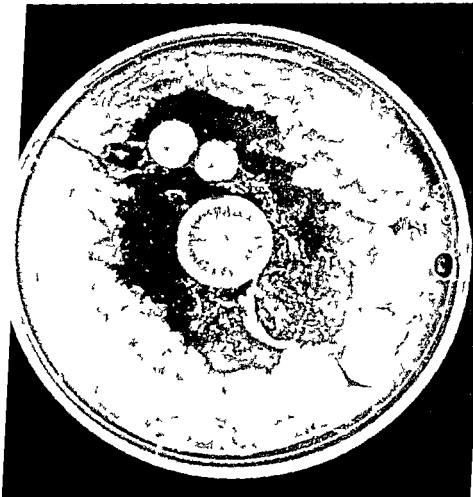


Photo from Merck & Co. Inc.

COLONIES OF *PENICILLIUM NOTATUM*

Dark area surrounding colonies is a zone of bacterial inhibition



PURIFYING CRUDE
PENICILLIN SALTS

Both photos from Merrick &



MAKING FINAL TEST OF THE
POTENCY OF PENICILLIN
BEFORE ANTIBIOTIC CAN
BE RELEASED FOR SALE

port (in *Lancet*), an American scientist took up the study of molds. Ardently and with remarkable patience Dr. Roger Reid, a bacteriologist at the Pennsylvania State College, investigated all sorts of mold organisms. Many different molds were tested, but Dr. Reid searched in vain. Under his test conditions none had the magic quality of Fleming's mold. None produced penicillin, not even those closely related. Dr. Reid's attempt was the only one to follow soon after Fleming's investigation. No new reports were published, no references to Fleming's work were made, either in scientific or in medical journals. For ten years deep silence shrouded this discovery.

What did Dr. Fleming do in the meantime? He struggled with the practical difficulties of research, which he was unable to overcome. Without the necessary funds, without the requisite facilities for this kind of work, he was fighting a losing battle. His first extracts of penicillin were not sufficiently stable and pure, nor were they very potent. The small amount of the drug which he was able to produce now and then was sufficient only to cover his requirements for experiments on animals. But he kept the culture of the original mold going during those years, although it required a good deal of work on his part.

On several occasions Dr. Fleming tried to interest his medical colleagues, but they turned a deaf ear to his unorthodox idea. He recruited only one ardent supporter, Dr. Harold Raistrick, of the London School of Hygiene and Tropical Medicine, a friend of long standing, believed as firmly as Fleming himself did in the potentialities of penicillin. Raistrick, a brilliant chemist and biologist, undertook the production of penicillin in small quantities. He made an intensive study to determine in which medium the mold would grow best. He tried to penetrate the mystery of this peculiar microorganism, to deduce the organic chemistry of penicillin.

When the two scientists, working separately, had at their

disposal a sufficient amount of penicillin to make a few clinical tests they approached their colleagues. Would you like to try our discovery on a few patients? they would timidly inquire of a prominent doctor. But when they explained that the drug was obtained from a common mold which had come out of nowhere to settle on Dr. Fleming's culture plate the practitioner flatly refused. After having discussed this matter with several medical men, Fleming and Raistrick gave up their attempt. Once more Fleming tried to appeal to the scientific world by reporting his discovery to a meeting of bacteriologists. His report was lost in a mass of other papers.

As each year went by his hopes diminished. Penicillin was on the verge of oblivion together with hundreds of other potentially useful discoveries when the Second World War broke out. Almost overnight the situation was reversed. Antibacterial remedies took the spotlight. Every product which might save the lives of wounded or reduce the high rate of death from infection received full attention and generous support. Thus penicillin was rediscovered.

The idea of an antibacterial agent was not very popular. It was still regarded as a stepchild of medical science but now more and more scientists turned to the problem.

Lysozyme was under intensive investigation and had even been isolated in crystalline form. If a germ-destroying substance is contained in human tears and in the white of egg there must certainly be other powerful germ-killing substances within or around us. But how to find them? Australian born Dr. Howard Walter Florey, professor of pathology at Oxford's Sir William Dunn School of Pathology and formerly a Rockefeller Fellow, was enthralled by this idea. He had at one time investigated the mysterious lysozyme, had published a paper or two on it and still considered it worth while to continue his research in this direction. He foresaw that this substance might turn out to be of great value. His

intuition was sound, as has been proven more recently in a fascinating manner by William L. Laurence of the *New York Times* and Professor Karl Meyer of the Columbia University Medical School.⁴

For years Dr. Florey's laboratory had attracted young scientists eager to work under his guidance. Scientists of many nationalities, American, French, Russian, and British were drawn to him. Two young scientists, Dr. Leslie Falk, an American, and Dr. Ernest Chain, a Russian, were engaged in research on lysozyme in Dr. Florey's laboratory when the subject of penicillin came to the fore. The war was approaching. The need for powerful antibacterial substances was of urgent practical importance. It was only natural that penicillin should be discussed at length in Florey's laboratory. They decided to recultivate the mold discovered by Fleming, the culture of which was still alive. The initial work on this subject was assigned to Drs. Falk and Chain. Later, the work was taken over by Dr. Florey himself; with the help of Dr. Norman Heatley and Dr. Chain, he brought it to a successful conclusion.

Needless to say, these men had at their disposal the excellent laboratories of the outstanding medical institution at Oxford. A grant from the Rockefeller Foundation, and another from the British Medical Research Council gave them the necessary funds to conduct the investigation on a large scale. More than twenty scientists and technicians assisted them. Pathologists, biochemists, bacteriologists, and physicians worked together in perfect coordination. The scope of their accomplishments was astonishing. In little more than a year they laid the foundation for penicillin therapy and established both the potentialities of the drug and its limitations.

Almost all the basic facts which the Oxford scientists established regarding penicillin were later confirmed by American investigators.

The problems we were facing were tremendous. Dr Florey later confessed. The journey from a test tube experiment in the laboratory to the actual treatment of the patient is always long and painful. Often it takes years before a new drug whatever its merits is accepted by the medical profession and administered to human beings. Penicillin was no exception. Before it could be tried on patients in a hospital there had to be assurance that it was nontoxic.

What is penicillin basically? It is a chemical substance produced by a mold which lives and grows in a specially prepared nutritive medium. When penicillin is extracted from this medium it is not pure. In fact when Dr Fleming first extracted penicillin it was very impure containing only an insignificant amount of the real penicillin.

The Oxford scientists had to purify the product as much as they could. Although they succeeded in increasing the concentration of the extract considerably over that of Dr Fleming's drug it was still far from perfect. For the first year or so they worked with an extract which contained hardly more than 5 per cent of penicillin in its pure chemical form. One gram of their drug contained no more than 50 000 units. By comparing these figures with the penicillin (average potency 9 000 000 units per gram) now being used in the United States it is easy to picture the difficult first steps of the British scientists. They found that penicillin by itself being a weak acid was unstable and easily destroyed by heat and air. They then prepared the salt (salts of sodium and calcium) of penicillin which was more stable and therefore easier to handle. Next it was necessary to establish some unit of activity for their drug as a basis for their tests that is they had to determine what was to be the unit of measure for penicillin. One unit was finally accepted as the amount of penicillin which would inhibit the growth of 2 500 000 streptococci in one cubic centimeter of medium. The Oxford

unit" is at present defined in terms of crystalline penicillin rather than in terms of microbiological effectiveness.⁵

What is the exact dilution of this drug, or the number of units, necessary to destroy disease-producing germs? How do the different types react to penicillin? The list of germs is very long indeed, and many are rare. The British scientists had to select the most commonplace germs, those which attack most frequently. They tested about eighty different types of microbes.

How did they try out penicillin on these germs? How did they determine which succumbed to the deadly effect of penicillin and which resisted its action? By test-tube experiments. They took small tubes containing 4.5 cubic centimeters of fluid medium and added 0.5 cubic centimeters of a weak solution of penicillin. Each solution was inoculated with a drop containing a specific germ and was left for twenty-four hours at 37°C. If the penicillin was effective no multiplication of microbes would be observed.

After repeating this test again and again with various types of germs, the experimenters arrived at the same conclusion as had Dr. Fleming. A number of germs are completely indifferent to even a strong dose of penicillin, while other bacteria perish at the merest trace of it.

The germ most sensitive to penicillin seems to be the one that causes gonorrhea. Even in a dilution of one part of penicillin to 2,000,000 parts of solution this germ does not multiply.⁶

Examining the germs that were placed in the test tube with penicillin, Dr. Florey and his co-workers came across a strange fact. They had expected the penicillin to kill the bacteria plunged into the deadly solution. But even the germs most sensitive to penicillin were not dead after remaining in the solution for twenty-four hours! True enough, the bacteria seemed apathetic, yet they were still alive. Nevertheless, they

had been unable to propagate. This discovery amazed the investigators. It seemed evident that penicillin was not an antiseptic in the strict sense of the word like a strong acid which kills germs even in a highly dilute form. Moreover it was not even a germ killing substance as are some of the more recently discovered drugs (like gramicidin).

It is not bactericidal was Dr. Florey's conclusion. "Bacteriostatic" is the term for a substance which immobilizes the germs by restricting their dangerous aggressiveness by making them impotent and unable to multiply. In his article reviewing his investigation of penicillin Dr. Florey pointed out, "It is bacteriostatic and not bactericidal" * at least in concentration likely to be used therapeutically and reliance must therefore be placed on the body defenses both humoral and cellular to destroy bacteria present in a lesion while penicillin prevents their multiplication. †

This fact having been established the next important question concerned the reaction of the human body to the drug. How would the living organism react if penicillin were introduced? Would the drug interfere with the normal defenses of the body? Or would it fully cooperate with the defense forces? On the answers to these questions depended the success or failure of penicillin as a therapeutic agent.

When germs succeed in penetrating the first line of defense of the organism—the skin or mucous membrane—a defense army at once rushes to block the invaders. The white blood cells, the leucocytes, the soldiers of our organism fight the intruders fiercely. The outcome of this struggle depends not only on the strength of the defenders but as often as not on the multiplication power of the germs. If the microbes are virulent and dynamic and grow rapidly the second line of defense may be pierced and the condition known as blood *infection may occur*.

* Actually penicillin is both bacteriostatic and bactericidal.

Penicillin may destroy most of the germs, perhaps as high as 99 per cent, providing, of course, that the germ is sensitive to the drug. Yet a few of the germs may escape destruction. These few, still in the system and very much alive, might be a source of potential danger to the organism as long as they are not destroyed completely by the white blood cells. How does penicillin affect the leucocytes? Does it immobilize them as it does the germs? The Oxford workers investigated this question promptly. They discovered that penicillin is practically harmless to the white blood cells. Even in a very strong concentration of 1:500, penicillin does not seem to impair the normal activity of leucocytes in the test tube; the white cells continue to move and live as they would in a nutritive medium without penicillin. Penicillin is, in fact, less harmful to the white cells under these circumstances than are the sulfa drugs, which have considerable inhibitory effect upon the activity of our defenders.

"It is clear," observed Dr. Florey, "that leucocytes will remain completely active in any concentration of penicillin likely to be reached after intravenous injection."⁸

When an infection takes hold, there is often a large quantity of the germ around the inflamed place, as well as a considerable amount of pus. How does penicillin work in these circumstances? Is it hampered by the concentration of the germs and by the presence of the pus, as is the case with the sulfa drugs, the effectiveness of which is decreased under such conditions? Not at all. Penicillin is as effective when there are only a few germs wandering around as when there is an army of many millions. Moreover, the presence of pus in the wound or in the inflamed area has no hampering effect whatsoever on this remarkable substance. The effectiveness of penicillin in the presence of pus or of a great multitude of germs is of tremendous practical importance, particularly in the local treatment of infected wounds.

Animal or human blood is not antagonistic to penicillin.¹ That fact is of primary importance. We know that whole blood has in itself slight antibacterial activity. But an addition of only 0.3 Oxford unit of penicillin per cubic centimeter of blood results in a striking increase in the antibacterial action. The activity of the whole blood containing only 0.07 unit of penicillin is much greater than that of whole blood containing 5.1 mg. of a sulfa drug (sulfadiazine) per 100 cc. of blood. These observations prove beyond any doubt that blood fluids are not hostile to the activity of penicillin, but rather seem to be an excellent medium for penicillin action. From the therapeutic point of view this is of utmost importance. Furthermore, British scientists established that diseased and even decomposed tissue does not impede the normal antibacterial activity of penicillin. Let us assume that we have an advanced case of cancerous growth which, in addition, is infected with some germ, as is often the case. Penicillin, although unable to cure the cancerous growth, might give considerable relief to the sufferer by destroying the infection. The presence of the tumorous tissue will not interfere with the action of the penicillin.

Dr. P. B. Medawar of the Zoology Department, Oxford University, demonstrated that penicillin is not harmful to the cells and tissues of the body. His technique consisted of utilizing living tissue *in vitro* (outside of the organism). Fragments of tissue from a ten-day-old chick embryo were grown in a nutrient medium to which penicillin was added in various concentrations. It was only when the drug reached a concentration as high as 1:1,000 that the growth was affected and the tissue began to show some signs of degeneration. However, in a concentration of 1:2,000 the tissues grew freely without apparent changes. The cells multiplied as usual, and their structure was not altered. A similar result occurred when the tissue of a mouse was placed in penicillin solution.

From this, as well as from the results of many other experiments conducted by the Oxford scientists it may be deduced that penicillin is harmless to the body tissues unless it is applied in a concentration higher than 1 1,200, which is never done in medical practice ¹⁰

Having concluded the first steps in their investigation, and being satisfied that penicillin is friendly toward the tissues and fluids of the human organism, the Oxford doctors now arrived at the next stage of their research. How long would penicillin remain in the animal or human system? They found that penicillin, if injected into the blood stream very rapidly disappeared from the system, being excreted in the urine. Their first test was with a rabbit. They injected penicillin directly into the vein of the animal. In less than one hour no trace of penicillin could be found either in the blood or in the organs of the rabbit. Next they used a cat. The cat retained the drug longer, but after three hours only an insignificant amount of penicillin was in the urine. A man who received a shot of penicillin in the vein showed a similar result. One hour or so after penicillin was injected, it began to disappear rapidly from the system and after two hours only a small amount could be found in the urine ¹¹

Thus, to keep penicillin in the system on a level sufficiently high to cure the infection it is necessary to give the drug every two or three hours without interruption, for the body does not retain it. It is like pouring water down a basin with the plug out, Dr Florey stated ¹²

Interestingly enough, the bile retains the drug the longest. As long as three hours after the drug is injected into the blood stream the bile will contain a very high concentration of penicillin (about five units per gram). 'Is there any practical consequence of this observation?' the experimenters asked. If the gall bladder were infected with a germ which is

antagonistic to penicillin in all probability the ailment could be relieved with this drug

Now arrived the most exciting moment in the investigation. All preliminary tests had proved quite satisfactory. Thus far, however, they had all been test tube experiments. How effectively would penicillin work on the actual battlefield—in the living organism? Many times previously scientists had been disappointed because their remedies, so powerful in the test tube, had been complete failures as disease fighters when introduced into the living organism. Would history repeat itself with penicillin? At last they decided to try the drug on animals.

Their first patients were eight white mice. The animals were infected with staphylococcus. When they became ill, the doctors began the treatment of four of the mice, injecting a small dose of the drug into their blood every three hours. The remaining four mice were left untreated as a control. The treated mice were quite ill, and the physicians were sure that the animals would not survive. But they continued the treatment, and gradually the first signs of relief became apparent. The animals no longer lay motionlessly, breathing hard. They attempted to stand up and walk about. After twenty-four hours of treatment, the mice were cured of the deadly infection.

We sat up through the night, injecting penicillin into the mice every three hours. Dr. Florey recounts: "I must confess that it was a most exciting moment when we found in the morning that all the untreated mice were dead and all the penicillin-treated ones alive."

The doctors were exhilarated. Here at last they had the first indication that the drug might work, not only in the test tube but in the organism as well. Now they were sure that Dr. Fleming's discovery had practical value. Now they were ready to experiment on man.

As narrated earlier, the first case treated with penicillin was a failure: the policeman eventually died from an acute infection of the lungs. The second case was a success. A boy suffering from blood infection was cured. Yet their third case, a four-year-old boy, was lost. Why? Was the dose of penicillin given insufficient, or the drug not as powerful as they had surmised? The post-mortem examination revealed that the boy had died of heart failure. The infection itself had abated, the germs having vanished under the influence of penicillin. The infection had been cured, when the boy died from exhaustion and resulting complications.

The case history of this little boy was tragic. He had contracted measles, which had cleared up, except for a slight sore on the eyelid. The same pus-forming germ that causes numerous other infections was responsible; the infection spread rapidly to the other eye and soon attacked the whole face. The child then developed an alarmingly high fever. Large doses of sulfa drugs were of no avail against the germs in his blood. He had already sunk to the state of semiconsciousness when, as a last resort, penicillin was used. The infection was arrested, and the fever went down—but the boy died. Assumedly, if penicillin had been administered at the onset of the infection, the child might have been saved.

The best method of treatment was found to be by intravenous or intramuscular injection. But why should penicillin not be given by mouth? It would be infinitely simpler to take in tablet form. Dr. Florey and his co-workers investigated the various methods of administration and observed that penicillin, being very sensitive to acid, was partially destroyed by the hydrochloric acid normally present in the stomach. Thus, if taken orally, the drug lost part of its germ-killing efficacy. According to them, if the patient's stomach acidity was low, or nonexistent, penicillin could be given by mouth

and with good results. To make their point the Oxford men cited a case which was successfully treated orally

The patient was a six month-old boy. The child had developed a diarrhea of uncertain origin and could not hold his food. The blood test showed a low red cell count of one and a half million while the high white cell count indicated the presence of an infection. Analysis of the urine showed staphylococci in great numbers. Evidently there was an infection of the urinary tract. Since the child was obviously in a dangerous condition the doctors decided to give him a sulfa drug which however had to be discontinued because the infant showed signs of mild poisoning. Penicillin was then given by mouth in doses of twenty milligrams each hour. After a few days of treatment the germ began to disappear from the urine and within two weeks the child had returned to normal.

Since the acidity in an infant's stomach is very low, the potency of the penicillin was not destroyed when passing through and was capable of exercising a curative effect. However in the mind of Dr. Florey the idea persisted that adults could also be given penicillin by mouth. He tried numerous methods of protecting the precious drug from the acidity of the stomach. On several occasions he administered penicillin in specially coated capsules insoluble in acid. The results were far from satisfactory. Then he made an attempt to introduce penicillin directly into the upper part of the small intestine (the duodenum) through a special tube. These efforts only proved that it is much more difficult to control the dose and the action of penicillin if given by mouth than if given by injection into the muscles or veins.¹²

Could penicillin be applied directly to an infected area? This was another question which concerned Dr. Florey. Together with Dr. Robert Williams he undertook an extensive investigation at Birmingham Hospital. They treated 212

cases of acute infection with penicillin. Their method was simple, and saved the hospital personnel thousands of working hours. The wounds or infected places were powdered evenly with calcium salt of penicillin (the sodium salt is irritating if applied locally), then were packed with gauze soaked in penicillin paste²⁴. The dressings were repeated every twenty four hours for at least five days.

Their results were most enlightening. The healing of the wounds progressed much faster with penicillin than if treated by the usual methods. In this respect the figures are striking. The wounds powdered with penicillin healed on the average in three or four days, while the control cases required six to nine days for recovery. In other words, the application of penicillin accelerated the healing to half the time. But even more important was the way the healing proceeded. Usually there was no pus at all, and the scar of the wound treated with penicillin was much smaller and less apparent than in the control cases. This observation, more than anything else, demonstrated the property of penicillin to cooperate fully with the tissues of the organism. Indeed, this drug almost seems mysteriously sympathetic to our natural healing processes. At any rate, it is not antagonistic, as most other drugs are.

Fifty five miles from the laboratory and clinics of the Oxford doctors the war was making a hell out of once peaceful London. For the Luftwaffe was attempting to raze the city. 'Is this the time to begin the production of penicillin in England?' the doctors wondered. "Will it be feasible, when all industries are occupied with the immediate needs of the nation? After some hesitation, Dr Florey and his associate Dr Heatley decided to accept the invitation of the Rockefeller Foundation to carry on their research in the United States.

At the time of their departure their clinical records were

still meager. Only five cases of blood infection had been treated with penicillin—two had failed, three had proved successful. Yet they were enthusiastically received by the American scientists. The work they had done was indeed valuable.

Here begins a new chapter in the saga of penicillin. Clinical investigations were begun on a large scale, and manufacture was undertaken on a mass-production scale. Two dozen large pharmaceutical companies simultaneously began to produce the new drug.

Nothing in the annals of medical science quite equals the startling and unusual story of the discovery of penicillin. The drug was on the verge of being completely forgotten. Then saved from oblivion by the war and rediscovered by a scientist of vision and dynamic personality, it proved to be a weapon of such magnitude and power that it may yet fulfill the dreams of generations of scientists.

6

Penicillin—Cinderella of Science

ON A HOT DAY in July, 1941, Drs Howard W Florey and Norman G Heatley, the two strangers from Oxford England, arrived in New York, in their possession the descendant of the mold which had been captured by Dr Fleming thirteen years before They were met by members of the Rockefeller Foundation

The British doctors discussed their problem with the members of the National Academy of Science and were referred to Dr Charles Thom, famous American mold specialist or mycologist, who was already familiar with their discovery He informed them that what they believed to be *Penicillium notatum* was not exactly that The penicillin producing mold was often referred to by the press as the green or blue-green mold found on bread, cheese, and other foods ' Dr Thom took pains to correct this This mold he said, ' is neither a cheese nor a bread mold, strictly speaking It is not even a typical *Penicillium notatum*, but it comes closer to it than to any of the other molds It is a member of the *Penicillium chrysogenum notatum* series, hence it appears in literature as *Penicillium notatum* " A fine point It is true that penicillin producing mold does grow on these foodstuffs at times, but

so do other blue-green molds which neither belong to the *Penicillium chrysogenum* group nor produce penicillin

Dr Thom who knows more about molds than anybody else in the United States warmly counseled the British scientists to go without delay to the Northern Regional Research Laboratory in Peoria Illinois to arrange for the production of penicillin

The Fermentation Division of the Northern Regional Laboratory is the most important center in the United States for research on molds For more than fifteen years small groups of scientists have been working there on the nutrition and cultivation of molds and bacteria searching for new methods by which to increase their rate of growth

The meeting between the Oxford men and the representatives of the Northern Regional Laboratory was the beginning of the American story of penicillin an extraordinary achievement of large scale production of penicillin The Northern Regional Laboratory deserves full honor for this project

Drs O E May and Robert D Coghill participated in the historic meeting A minor difference in the points of view of the scientists was a matter of pronunciation Dr Florey and his colleagues said penicillin The Americans called the drug penicillin However this slight difference did not prevent a complete understanding

They sold us the problem Dr Coghill said He and his associates realized the enormity of the task of organizing the production of penicillin in appreciable amounts How much penicillin would be needed to test the drug clinically on a limited scale? The answer was one kilo of crude penicillin At first that appeared to be a small amount Actually it was a huge quantity almost unobtainable by the technique the British workers had used The commercial companies that had shown some interest in the discovery of penicillin such as Merck Squibb and Pfizer hesitated to start production

because the yield of penicillin seemed too low to enable them to achieve reasonably large production, even in the distant future. The first and most urgent problem was to increase the yield of penicillin from the mold. But how was it possible to force the production in greater quantities? After many months of research the problem was solved.

PRODUCTION OF PENICILLIN

This mold, like any other, goes through a reproductive process in which the microorganism becomes covered with tiny spores. The spores are needed for further cultivation of the mold, but while the spores are being formed, the mold does not produce much penicillin. Only during the period of its growth is penicillin formed. Thus these two periods in the life of the mold require separate handling. The spore-producing cultures are placed on an agar slant made in a small flask. Usually, but not necessarily, the flask is put in a screw capped bottle. In due time the material from these small flasks is transferred to larger flasks or to bottles, and in this manner the spores are collected for inoculating the media on which the mold is to be grown. When a stock of spores is available, the manufacture of penicillin can proceed.

From study of the physiology of this mold it was learned that the mold should grow in shallow, flat vessels or tanks in order to be in constant contact with oxygen, and that it prefers a not-too-warm temperature. It does not grow well at body temperature (98°F. , or higher) and is at its best at a warm room temperature of 75°F. (24°C.).

Twenty-four hours after the spores have been planted in the flask of sterile nutritive medium, a very delicate, fluffy and gauzelike growth can be detected on the bottom of the vessel. The growth expands during the next day. On the third day it extends to the surface and throws out a dry,

white mycelium (a network of fine threads) particularly around the sides of the vessel. Usually by the fifth day the whole surface of the medium is covered with the dry mycelium which soon begins to turn bluish green. By about the seventh day the growth consists of a continuous compact and often corrugated dark greenish blue felt the upper surface of which is water repellent. The under surface is of course freely wetted and is brownish yellow and slimy. Even before the mold has reached its peak of growth a faint yellow color can be observed in the medium. The yellowish solution contains the penicillin.

To some extent the problem of mass production was solved at the beginning of 1942. As the methods were improved the number of chemical companies engaged in the production of penicillin gradually increased. By 1944 twenty-one concerns had an investment here of nearly \$20 000 000. Thus American know-how helped to speed the manufacture of this drug on a scale large enough to supply human needs.

However the problem of production was only partly solved for the peculiar characteristic of Fleming's mold is that it does not want to submerge. It grows more freely and actively on the surface of the medium. Attempts to use large tanks for its growth under the surface (we call it deep fermentation) were only partially successful and met with many difficulties in the beginning. The cultivation of the mold in flasks a method used by Dr. Florey and by some of the chemical companies has two serious objections. It requires large space for mass production and a high labor cost. To process only one thousand gallons of medium which in terms of pure penicillin is a small amount requires as many as 10 000 to 12 500 bottles a large number indeed to be cleaned and washed and checked.

Dr. Coghill decided to seek another mold which could be cultivated in submerged fermentation. Fortunately among

their collection of molds his collaborators found a strain of *Penicillium notatum* which was able to produce as much penicillin as did Fleming's mold.¹ The most amazing thing about this second mold was that it lent itself to submersion into the medium and would grow deep under the surface. In fact, this strain grows much more slowly on the surface. It can be successfully cultivated in large vat fermenters or rotary drums with aerators, under conditions which supply a constant amount of absolutely sterile air.²

Thus there are now two strains of molds, both of which are used for the manufacture of penicillin under different physical conditions. The first mold, the descendant of Fleming's mold, used for the *surface culture method*, is known as NRRL 1249, B21. The second mold, NRRL 832, serves for the production of penicillin by the *submerged culture method*. By the latter method the mold grows in small pellets, rather than in the heavy pellicles which are produced in the surface culture method. When cultivated in large vats, the yield of penicillin is as high as a hundred units of the drug per one cubic centimeter of filtrate. The time of production is also shortened; in two or three days the maximum yield may be obtained.

THE ROLE OF NUTRIENTS IN PRODUCTION

Various nutritive substances affect the growth of the mold. This fact was first observed by Fleming who found that the mold flourished in trypsin-digested broth. In the beginning glucose was used as an essential ingredient of the medium, but later C. M. McKee and G. Rake recommended the substitution of brown sugar, because it contains an organic substance which stimulates the production of penicillin. Other investigators found that small amounts of zinc sulphate (J. W. Foster³), and manganese (W. Kocholaty⁴), promote the growth of the mold. But the most important discovery in

this respect was made by Dr. A. J. Moyer, a microbiologist working with Dr. Heatley. His finding rendered more feasible the production of penicillin on a commercial scale. In studying various materials as possible food for the mold, Moyer noticed that as soon as he added a small amount of corn steeping liquor to the medium, the yield of the precious drug would rise sharply. Instead of an average yield of five or six units per cubic centimeter of medium, Moyer would recover about two hundred units per cubic centimeter. Thus through his discovery the yield of penicillin was increased fifteenfold or more.

PURIFICATION OF PENICILLIN

The proper cultivation of the mold is only the first step, although an essential one, in production. The manufacturers were confronted with a no less difficult problem in the extraction and purification of the drug. After five to seven days of producing penicillin energetically, the mold becomes exhausted, and no longer capable of yielding any appreciable amount of the drug. That is the time to extract the penicillin from the medium. After the mold has been removed from the medium by filtration, a yellowish liquid is left behind. How much penicillin does this liquid contain? Not much, in spite of all the painstaking methods. By weight this solution contains no more than 0.03 per cent of penicillin. Roughly speaking, sixteen liters of medium filtrate yield one gram of penicillin. Small wonder that Dr. Coghill once remarked: "The recovery of penicillin is very much like looking for a very unstable needle in a hay stack." The recovery is further complicated by the instability of this drug. The extraction must therefore be carried out at a low temperature and as quickly as possible.

In their laboratory experiments, the Oxford scientists extracted penicillin by means of ametyl acetate, an organic

solvent, using a continuous-countercurrent extraction apparatus⁶ In large scale production absorptive processes are employed in the first concentration But that is only a preliminary step in the actual extraction Penicillin must then be removed from the absorbent and concentrated further The simple and effective butanol-petroleum ether method is often used At this point the drug still contains some proportion of water Since penicillin is an unstable product in solution, a complete dehydration is imperative, yet heat cannot be applied The delicacy of the drug requires that its solution be concentrated at low temperature by evaporation of water from the material in a frozen state Steam ejectors, mechanical pumps, and a freezing method dry penicillin in a vacuum The technique is somewhat similar in principle to that of drying blood plasma In fact, like blood plasma penicillin can be stored without deterioration only when moisture is completely, or almost completely eliminated No more than 0.5 per cent of moisture is allowed The most difficult part in this dehydration is the elimination of the last 2 or 3 per cent of moisture It takes as much time to drive off this last 2 per cent of moisture as to eliminate the first 96 or 97 per cent.

Usually a twenty-four to forty-hour cycle is required to accomplish this very expensive job Some chemical companies use dielectric heating at radio frequencies This method, developed by the Radio Corporation of America, is very fast The bottles are rotated at high speed and the penicillin solution is held by the centrifugal action to the walls of the ampoules until the drying is completed

Penicillin can be obtained in the form of free acid or in the form of various salts At the present time only two forms of penicillin are generally employed—sodium and calcium salts The purification of this drug has been so perfected that the sodium or calcium salts may contain as much as 5,000

to 9 000 units of penicillin per milligram of dried material.

Penicillin in the form of calcium or sodium salts is relatively stable toward air. When left exposed it retains its potency for a considerable length of time if the temperature is low, but if kept at a temperature of 96-98°F for a few days it may lose all its potency. When penicillin tablets are kept in a refrigerator the drug remains unchanged for three months or more. However, if no refrigeration is available penicillin tablets may safely be kept for a day or two in a cool place where the temperature is no higher than 20-25°C.*

Penicillin solutions may be stored in the refrigerator for as long as two weeks. But if the drug is left for a longer time even on ice it may lose some of its potency. The drug is very sensitive to acids; if taken by mouth penicillin tablets should not be dissolved in orange or grapefruit juice. It may be better to swallow them in milk. This gives the drug some protection against the hydrochloric acid in the stomach.

Several attempts have been made to prepare penicillin in a more stable form which would successfully resist the detrimental effect of stomach secretions. The Oxford workers were the first to try it with poor results. Dr. Karl Meyer and Dr. Gladys Hobby of the College of Physicians and Surgeons, Columbia University, New York City, were much more successful. They prepared the methyl and ethyl esters of penicillin. Not only did these preparations possess a higher antibacterial activity than the salts of penicillin but they also exhibited considerable stability. These compounds could be given by mouth to experimental animals and still maintain the penicillin in the blood on a high level.⁷ C. J. Cavallito and his associates produced another compound which they claimed to be of even higher stability, benzyl ester of penicillin G. It is obtained in the form of a colorless sticky solid which remains stable at a temperature as high as 100°C. When taken by mouth this compound appears to be

about three times as effective as the regular tablets of the sodium salt of penicillin ⁸

CHEMICAL NATURE OF PENICILLIN

At first it was believed that the mold *Penicillium notatum* produces only a single form of penicillin. But as the scientists began to penetrate the chemistry of this substance they found that there are actually four types of true penicillin produced by the mold, in addition to some penicillin like compounds. These types have been identified and named as penicillins F, G, X, and K. In England they are called respectively I, II, III, and IV penicillins. Penicillin produced by the surface culture method is largely of type F, while type G is found mostly in the submerged culture of the mold. Therefore, the commercial penicillin is composed largely of penicillin G, although one may find a great variety of penicillins on the market. There seems to be only a slight difference between these types of penicillin so far as their therapeutic action is concerned. Penicillin X is presumed to be less effective than the other types when taken orally ⁹. On the other hand, penicillin X has been found more active against many bacteria than penicillin G. Penicillin K, however, seems to be of little value in the treatment of syphilis.

Many efforts have been made to identify the chemical structure of penicillin but for a long time these efforts were fruitless. Recently the specifications were at last established. The drug is of relatively simple structure but possesses an entirely novel type of molecule—a dipeptide of a special type. The chemical structures of the four types of penicillin differ not in the nucleus which is common to all penicillins but in the side chains. The striking feature of the penicillin structure is the four member β -lactam ring. Such a structure has so far not been disclosed in any other known biological compound and it has never been produced syntheti-

cally As a matter of fact in spite of the simplicity of the chemical structure of penicillin no method has yet been suggested for producing the drug synthetically

Besides the four types of penicillin which have the same common nucleus and which act in a similar if not identical manner against the germs a penicillin like substance has been found in the culture medium of the mold.

The discovery of this new substance quite by accident, is a story in itself In 1941 Dr Edward Doisy Professor of Biochemistry at St Louis University and famous for his work on hormones decided to concentrate on penicillin For his work he procured a strain of the identical mold with which Dr Fleming had been working He used almost the same medium as Dr Florey's. However there was a small modification in the nutritive medium which made the difference When Doisy and his co-workers began to extract the drug, they found to their bewilderment that the mold was producing a substance quite different from Fleming's product for while the original penicillin can be extracted by ether and amylacetate Doisy's drug was insoluble in these solvents It had to be separated from the culture medium by absorption with benzoic acid to which it responded readily Further more Doisy discovered that his product was much more stable than penicillin In dry preparation his penicillin was a yellowish hygroscopic powder completely soluble in water and stable for months In fact it retained its activity (in powdered form) for at least six months Realizing that he had a new product Doisy called his drug penicillin B It was soon established that penicillin B exercised its deadly effect not only on the bacteria of the Gram positive group as does Fleming's penicillin but on some of the bacteria of the Gram negative group as well ¹⁰

The chemical structure of penicillin B contains three benzene rings joined together with one atom of nitrogen in each

ring Unfortunately, it was soon proved that this twin brother of penicillin, while very effective in the test tube, had little power to combat germs when introduced into the system Penicillin B affects bacteria indirectly It interferes with bacterial respiration, but, instead of cutting down the supply of oxygen, penicillin floods them with it Just as too little oxygen is bad for both bacteria and man too much is equally damaging, perhaps even more dangerous The excess of oxygen causes the body's energy fuels to burn too fast and in the end brings about a complete breakdown of the delicate system of respiration and oxidation

As Dr Doisy demonstrated, penicillin B exercises its anti bacterial power by oxidizing glucose, the simple sugar present in the body of man as well as in bacteria and by producing hydrogen peroxide, a familiar antiseptic This literally burns the bacteria alive Unfortunately, there is a factor in the blood and tissues which counteracts the paralyzing effect of penicillin B on bacteria An enzyme called catalase, neutralizes the antibacterial action of this drug by decomposing the hydrogen peroxide as fast as penicillin B produces it Man's organism will not tolerate the presence of hydrogen peroxide for any length of time This reaction in the living body is so definite that it leaves little, if any, hope that penicillin B will be widely used in medical practice

Astonishingly enough, the paradoxical penicillin B was simultaneously discovered in three other laboratories in different parts of the world Each of the discoverers gave a different name to this second substance, produced from the original Fleming mold In Australia Miss Nancy Atkinson called the new substance, rather melodiously, *penicidin* In England, Dr H Raistrick isolated a similar substance and called it *notatin* And Dr W. Kocholaty in the United States gave the most detailed description of a new substance which he obtained from a similar mold which he called *penatin* At

first these discoveries provoked a mild confusion, but when the scientists compared notes on the action of the twin brother of penicillin they agreed that they had all found the same substance. It is difficult to determine who was the earliest to find this additional drug produced by the famous mold. These scientists all published their reports at about the same time. In any case, penicillin B seems to be identical with penicidin, notatin, and penatin.

PENICILLINASE

In many instances when penicillin is taken by mouth its effectiveness is greatly decreased. Even in the most stable form, which is designed to resist the destructive action of the gastric juices, penicillin seems to be largely destroyed in the intestinal tract. This is due to the presence of the bacteria inhabiting the intestinal tract.

Still another question confronted workers investigating penicillin. Why was this drug so ineffective against the Gram negative group? Why had penicillin even in infinitesimally low dilution, the miraculous power of destroying staphylococci and streptococci, but was almost powerless even in concentrated form, against *Escherichia coli* or *Shigella dysenteriae*? Drs. E. P. Abraham and E. Chain seem to have found the answer. According to them, some bacteria of the colon typhoid group produce an enzyme which can be easily extracted and isolated and which is destroyed by slight heating. This enzyme—called *penicillinase*—is antagonistic to penicillin and is in fact, able to destroy it. Thus if penicillin is added to a test tube containing the culture of *Escherichia coli*, not only will the germs remain alive, but the drug will gradually be decomposed by the enzyme which they produce. Identical processes take place within the human body. Even very strong doses of penicillin are unable to exercise their destructive power upon the colon bacilli. The latter

emerge victorious, for they have at their disposal a much stronger weapon than the one produced by the mold. In deed, if an agar plate containing cultures of the colon bacillus is contaminated with the mold *Penicillium notatum*, in the fight for survival all the advantage is on the side of the germs.

The colon bacillus is not the only one able to withstand penicillin. There are some strains of staphylococci which also resist its action. Dr. William Kirby, of the Stanford University Medical School, decided to investigate these stubborn germs and isolated several different strains of staphylococci. Preparing an extract from them, he found that all contained a substance that neutralizes, or inactivates, the action of penicillin. Those staphylococci which resist penicillin do not produce penicillinase. Their resistance, therefore, is based on a different principle. They simply adapt themselves to the destructive action of the drug.

Further investigations in this direction have shown that although some bacteria, like *Bacillus subtilis*, do produce penicillinase, the germ is yet susceptible to the drug to a moderate degree. Summing up these results, it is apparent that the bacteria capable of producing the antipenicillin substance are much less susceptible to its action.¹¹

One question still remained unanswered. Exactly how does penicillin destroy germs? By what physiological means is this drug capable of inhibiting the growth of the most aggressive germs without damaging the tissue and organs of the body? An intriguing theory was suggested by the scientists working on the problem.

The bacteria that attack human or animal organisms and cause various diseases depend on a proper and uninterrupted supply of oxygen and other nutrients. To live and propagate they must breathe and eat. Although their physiology and their use of nutritive substances differ greatly from those of

the living cells in many instances both the bacteria and the cells of the organism use the same nutrients. If the germs are deprived of essential foodstuffs without which they cannot exist or propagate, obviously their aggressiveness will be broken down.

According to the workers of the Iowa State College penicillin does not kill the bacteria directly but deprives them of the food they need. Dr. H. J. Weisheimer and his associates have described the process by which they maintain, penicillin is capable of producing the lethal effect on so many germs without causing harm to the human organism. One of the products of our metabolism is a substance known as pyruvic acid. For both the living cells of our bodies and the germs that invade them, pyruvic acid is an essential nutrient in their complicated metabolic processes. Without this substance they can hardly exist. But if both the human organism and the germ need pyruvic acid, how by depriving them of this acid can penicillin destroy the germs without harming the cells of the organism? The truth is that the germs and the living tissue consume this substance in different ways so that, while penicillin obstructs the use of pyruvic acid by the germs, it does not interfere with the utilization of this substance by the human body.

Deprived of this foodstuff and unable to replace it with any other nutrient, the germs are starved out. They may continue to lead an apathetic existence, but they do not have the energy and vitality to propagate or to become aggressive. Figuratively speaking, the drug cuts the lines of supply to the army of invaders and thus prevents, or makes impossible, their offensive drive.

Other workers offer a different interpretation of this drug's action. G. L. Hobby and M. H. Dawson, as well as G. P. Miller and A. Z. Foster, expressed the opinion that penicillin is particularly effective against the bacteria when

they are in a state of propagation, by obstructing the oxygen intake of young germs¹²

In his original work Fleming observed that staphylococci growing near the mold on the agar plate became transparent and underwent disintegration (lysis). He therefore believed that penicillin acted directly upon germs, that it was bactericidal. The Oxford workers, however, inclined to the opinion that penicillin acted indirectly by inhibiting their growth and was therefore bacteriostatic. The general consensus of belief at present is that the drug acts both directly and indirectly on the germs. Thus *penicillin is a bactericidal as well as a bacteriostatic substance*¹³. For precisely this reason, it must be regarded a truly magic drug.

Only eight years have passed since that memorable meeting in the Peoria laboratory, but the accomplishment should be measured in decades. The production of penicillin which was started under the most difficult circumstances, has already reached a gigantic volume. Moreover it is based on sound premises with every hope of future extension and improvement in the cultivation of the molds. Our knowledge of the mysterious mold itself has increased, and science is gradually beginning to understand the very complicated mechanism of its production machinery. Penicillin has already saved hundreds of thousands, if not millions, of lives.

Moreover, the progress made with penicillin has given new impetus to research in the chemistry of molds and bacteria, a field not too popular until a decade ago. A new science—the science of the domestication of microorganisms—is being born, and already is rapidly extending its activity. New horizons are opening in our search for antibacterial substances. What is the future of penicillin, that "Cinderella of science," as Dr. Coghill so appropriately called this drug? What is the next step in its research? Will this drug be synthesized? Are we on the way to the final discovery, which

may open new possibilities for its production and therapeutic application? Penicillin has not yet been synthesized, but one cannot help agreeing with Dr Fleming, who, in a speech broadcast from London on December 13 1943 said

What now remains is the synthesis of penicillin and this has a much wider significance than just an increase in production. The chemists will fasten on the molecule and modify it as they have done with the sulfanilamide molecule in the last five years so that derivatives of penicillin will appear more powerful or with wider application and diseases now untouched will be conquered.

7

Penicillin—an Antimicrobial Agent

DR FLOREY'S sojourn in Peoria was brief. He was encouraged by the enthusiastic reception on the part of the laboratory workers and was impressed by their scientific activity and extensive knowledge in the field of fermentation in general and of molds in particular. He departed for Washington assured that the problem of penicillin production was in the right hands. Dr Heatley, his associate, remained in Peoria to assist in the work and to introduce to American workers the intrinsic mechanism of Fleming's mold.

In Washington Dr Florey met Dr A. N. Richards, vice president of the University of Pennsylvania and in charge of medical affairs there. A man of vision and initiative, Dr Richards lent Dr Florey his full support. In fact, to him is due a good share of the credit for the emergence of penicillin from the status of a research product to large scale application. Through his efforts the large drug concerns received financial backing and orders from the government, so that they were enabled to undertake the manufacture of penicillin in quantities sufficient to save the lives and protect the health of the men in the Armed forces.

As the Chairman of the Committee on Medical Research

in the Office of Scientific Research and Development, Dr Richards was in an excellent position to start things moving in the right direction. Penicillin needed to be under the aegis of some important organization, which could supervise production and provide the funds. He brought penicillin production to the attention of the OSRD, a government bureau set up by the President in 1943, within the War Production Board to promote inventions of use in national defense. At no other time in the history of the United States had the national government taken so active an interest in a medical discovery.

As soon as production was under way, and a constant though limited supply of penicillin assured, Dr Chester S. Keefer, of the Evans Memorial Hospital in Boston, was invited by the OSRD to organize the clinical investigations in several selected hospitals. A group of prominent medical men in various parts of the country were accredited to investigate the therapeutic value. They were supplied with limited amounts of penicillin and were required to report their results promptly.

The selection of Dr. Keefer as head of the clinical investigation on penicillin was excellent. For years he had been interested in the antibiotic substances and together with Dr. Charles H. Rammelkamp, had made a number of investigations on this subject at the Evans Memorial Hospital.

In the meantime, however, before penicillin was available from the chemical companies, a few doctors with the pioneer spirit had initiated their own independently conducted investigations of the drug. Theirs was not an easy task. They succeeded in growing the mold obtained from Dr. Fleming in hospital laboratories and went through the painful procedure of extracting and purifying the crude material. They followed, more or less, the technique described by the Oxford workers and produced penicillin of considerable strength,

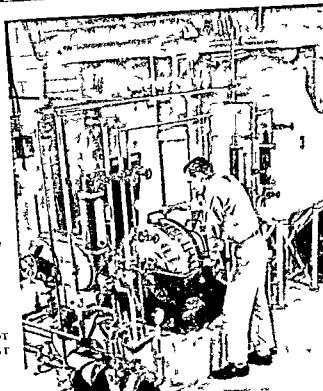


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LARGE EQUIPMENT USED FOR EXPERIMENTS ON ANTIBIOTICS

but in very small amounts. The first physician in this country who had the courage to test the drug at that time completely new and untried, was Dr. Martin H. Dawson of the Columbia University Medical School. The Mayo Clinic followed. The first reports by Drs. F. R. Heilman and W. E. Herrell of the research staff of the Clinic were enthusiastic but they had such a limited supply of the drug that their clinical investigations were jeopardized as had been those of the Oxford doctors, they had to limit their experiments to a few patients often finding it necessary to administer too small a dose to be completely effective.

When Dr. Florey arrived in this country there was considerable enthusiasm on the part of a few leading men but the majority of the profession was skeptical about the 'strange drug' extracted from a mold. Their skepticism was quite understandable. The clinical data brought by the British scientists was meager and far from complete. But as the evidence accumulated enthusiasm for penicillin snowballed. Today there is not a physician or surgeon who will not prescribe this drug to his patients with full confidence. Penicillin is the best friend of the practitioner, an aide on which he can depend in serious cases of infectious disease. In recent years much has been learned about both its limitations and its potentialities. Millions have been treated with it, and the clinical as well as the experimental data is truly enormous.

PENICILLIN VERSUS BACTERIA

Through centuries of contact with man most of the disease-producing germs have adapted themselves to existence in the bodies of human beings. Some of these germs are called true *pathogens*. These germs are able to invade the tissues of healthy persons through some peculiar power of their own. There are not many species of true pathogens. Each causes a

typical illness such as tuberculosis or syphilis. Many other types of germs may be called opportunists. They are harmless as long as they cannot penetrate into living tissue and break the line of defense of the organism.

One of these bacteria is the common *Staphylococcus aureus* and another is *Streptococcus viridans*. They are found on the skin and are present on the tonsils or in the nasal cavity. In fact these two typical opportunists may be found on every healthy person almost everywhere on the surface of the human body. These germs given the opportunity may cause the most dangerous cases of blood infection possibly complicated by an infection of the inner lining of the heart. These germs cause abscess of the cheek or a furuncle or an infection of a tooth.

Fleming himself was the first to prove that *Staphylococcus aureus* is highly susceptible in the test tube to crude penicillin. This observation was confirmed by many other workers (Abraham *et al.* Rammelkamp and Maxon) but when various strains for there are numerous strains of this germ had been tested it was disclosed that not all staphylococci are sensitive to the drug. Dr. W. W. Spink and his associates examined sixty-eight different strains of *Staphylococcus aureus* and found that 12 per cent were resistant to penicillin.¹ Although these tests were made *in vitro* they seemed to correspond to similar though not identical figures obtained in animal experimentation and in clinical evidence. Thus when there is an infection caused by this germ the chances are one against nine that penicillin will be powerless to arrest it.

The streptococci vary greatly in the degree of their susceptibility to penicillin. Some of them fall easy prey to this drug while others show considerable resistance. *Streptococcus hemolyticus* a blood splitting germ is very sensitive to the action of penicillin. Unfortunately *Streptococcus viridans*

the dangerous germ which so often causes the infection of the lining of the heart (subacute bacterial endocarditis) is more resistant to penicillin. In general, the group of viridans, or green-producing streptococci, succumb to the destructive action of the drug only when a large dose is administered.²

Experiments on animals confirmed these experiments in the test tube. Dr. E. Jawetz, from his experiments on mice, arrived at the conclusion that the success of the penicillin treatment depends greatly on the quantity of the drug given and the length of time since the beginning of the infection. If penicillin is administered at the onset of the infection, the results are quite satisfactory. But if the infection has become advanced the outcome of the treatment may not be so favorable.³

A group of streptococci, known as anaerobic hemolytic streptococci, often causes infection of the lungs and has been proven very resistant to penicillin. If such a germ is detected as the cause of pneumonia it is imperative that large doses of penicillin be given. Otherwise the illness may be fatal.

The pneumococci, which in the past were the cause of so many deaths from pneumonia, are extremely susceptible to penicillin. All types of pneumonia diplococci are easily destroyed, although some variation in the effectiveness of the drug has been observed. Mice infected with pneumococci of various types were well protected against the infection by the administration of relatively small doses of penicillin. Even when the drug was given to mice by mouth the treatment was most effective,⁴ for this type of germ does not have a natural resistance to the drug, as do some of the staphylococci and a few of the streptococci.

Neisseria gonorrhoeae, the germ which causes gonorrhea, is highly responsive to penicillin; other bacteria of the same

family are somewhat less susceptible. Gonococci do not possess a natural resistance to penicillin so that a proper dose of the drug easily liquidates them.

The small cocci known as meningococci which are responsible in most cases for the grave disease meningitis (inflammation of the brain membrane) are also very susceptible to penicillin. Not only in the test tube but in animal experiments as well they were destroyed by this drug. Apparently most types of *Neisseria meningitidis* respond promptly to treatment with penicillin in experimentation.⁶

Lockjaw or tetanus is caused by a germ called *Clostridium tetani*. It is a small slender bacillus which can be found in the superficial layers of the soil. Manured and cultivated earth usually harbors this organism which can cause a fatal infection in man. Experiments have proven that it is highly susceptible to the action of penicillin. The other species of the genus *Clostridium* also are strongly affected. The drug is highly effective against *Cl. welchii*, *Cl. histolyticum* and many others. Penicillin also wields considerable power against diphtheria germs. *Corynebacterium diphtheriae* and other diphtheroid bacilli are equally susceptible to penicillin.⁶

No one foresaw that penicillin might be used against syphilis but its effectiveness is now beyond doubt. The story of the discovery of this striking property of the drug was related by Dr. John F. Mahoney at the conference of the American Public Health Association in New York in 1943. The report drew the attention of the entire medical profession.

A few months earlier Dr. Mahoney and his associates had begun to investigate the effect of penicillin on gonorrhea. In their laboratory they had a number of rabbits infected with *Treponema pallidum* the germ of syphilis. Purely as a gamble Dr. Mahoney tried the penicillin on these rabbits. He was fairly certain that it would be fruitless for the experi-

ments in the test tube by McKee and others had not been encouraging.⁷ Thus there was no reason to expect penicillin to work on animals infected with the spirochete. Moreover, Dr R C Arnold, Dr Mahoney's associate, had himself attempted without success to destroy with penicillin the spirochete in the test tube. But Dr Mahoney persisted. At his instructions, Dr Arnold injected a rather large dose of penicillin into the ear vein of a rabbit. Before the injection the rabbit's ulcer was full of syphilitic germs.

Although he was confident that there could be no change in the sick animal's condition, Dr Arnold a few hours later reexamined the rabbit's syphilitic ulcer. To satisfy my chief,⁸ he recalls, "To his surprise he discovered that most of the spirochetes had disappeared. 'Perhaps there is some mistake,' he said. However, six hours later the ulcer of the rabbit was completely free and not a single spirochete could be found there.

Excited, but still doubtful, Dr Arnold reported his findings. Dr Mahoney's first reaction was one of doubt. The news was too good to be accepted without reservation. Certainly no one could expect that penicillin would kill *Treponema pallidum*. Quite often some drug proved effective in the test tube and useless when applied in vivo on animals. But very rarely was the reverse situation true.

The doctors repeated their experiments on rabbits again and again. The results were always the same. With clocklike precision all germs in the blood of the animals were annihilated within ten to twelve hours after injection of the drug. This finding by Drs Mahoney and Arnold opened the doors for the clinical treatment of syphilis in man with penicillin.

Numerous other investigators have confirmed their work. Harry Engle and his associates, W C Raiziss and others, have reported that injections of penicillin in large doses have proven completely curative in the treatment of syphilis in

rabbits.⁸ The consensus is that penicillin, given in proper doses over a period of eight days is capable of completely curing experimental syphilis in rabbits.⁹ Moreover, if injected shortly after infection a single large dose is sufficient to clear up the disease.

Penicillin is effective against other types of spirochetes, including *Treponema pertenue*, the agent of a disease similar to syphilis known as yaws. There are indications that the drug is as effective here as against syphilitic spirochetes.

Another spirochete bearing the romantic sounding name of *Borrelia novyi* and responsible for the infectious disease of relapsing fever is very susceptible to penicillin. Heilman and Herrell have reported convincing results on mice inoculated with *Borrelia novyi*. They used two batches of mice, each containing twenty seven animals. The control group was infected with the germ of relapsing fever, but was left untreated. Twenty-one mice or 75 per cent, died promptly. The mice of the other batch also infected with the same germ were treated with penicillin for four days. All the mice, except one, survived and were completely cured of the infection. Penicillin is also effective against many other spirochetes (*Leptospira Spirillum*, etc.)

Penicillin shows very little activity against the group of Gram negative microorganisms. The bacteria which cause various intestinal infections such as *Escherichia coli*, *Bacillus typhosus*, *Salmonella paratyphi*, *Vibrio cholerae* show considerable resistance to the drug in the test tube. Although in high concentration penicillin is capable of producing a slightly inhibitory action on some of these germs it is assumed that the therapeutic value against these germs is very small. Consequently almost no experiments on animals were conducted with this type of germ. *Brucella abortus* and *B*

⁸ For present status of penicillin in treating syphilis in human beings see pages 153-155.

melitensis, two species of the genus responsible for undulant fever, are also highly resistant to penicillin.⁹ Rickettsiae, the germ causing Rocky Mountain spotted fever and typhus fever, are moderately inhibited in vitro by penicillin, but the experiments on animals have given negative results.¹⁰

What effect has penicillin on virus organisms? The virus of poliomyelitis is completely indifferent to the drug. Even very large doses produce no inhibiting effects. The same is true with the virus of smallpox and of encephalitis.¹¹ However, penicillin may have some inhibiting action against other viruses, such as the one which causes parrot fever. Heilman and Herrell, of the Mayo Clinic, recite the interesting characteristics of these germs, and report their attempts to cure the disease in animals with penicillin.¹²

In 1892, a mysterious disease broke out in Paris. Hundreds of persons died from a grave infection of the lungs. The epidemic was traced to a peculiar incident. Five hundred parrots had been imported from Buenos Aires to Paris. En route about three hundred parrots had died of an unknown infection. The episode might have passed unnoticed, except that one of the importers also contracted the disease and succumbed. His brother, affected with the same ailment, survived, but on his return to Paris many of his neighbors and friends fell victim to the germ.

The disease was named *psittacosis*, or *parrot fever*. Subsequently, a germ was isolated and identified as a virus-like organism. The germ was infectious for parrots, hens, and budgerigahs. From time to time epidemics occurred in various European countries, as well as in the United States. During the winter of 1929-30, one hundred sixty-nine cases of parrot fever were recorded here. About 20 per cent of those infected died.

When the scientists investigated they decided that not only were parrots a source of infection for men, but canaries and

pigeons as well transmitted the disease Dr K. E Meyer showed that pigeons which might appear perfectly healthy were possible carriers of this germ According to him, as high as 60 per cent of pigeons in some breeding lots will yield the virus of the infection Since there are so many hosts for this germ, Meyer suggested that the term *psittacosis* should be applied only to the infection caused by the virus transmitted by parrots The term *ornithosis* is used when the source of infection is of another species

There have been many cases of pneumonia which have been diagnosed vaguely as "virus pneumonia" or pneumonia X but which were actually incited by the virus of parrot fever, or pigeon fever For this reason, considerable importance is attached to the finding of an effective method for the treatment of these diseases

The workers of the Mayo Clinic secured a germ from an infected pigeon With this deadly pigeon virus they inoculated two batches of young Swiss mice, totaling eighty animals Seventeen hours later half of the mice received injections of penicillin The treatment continued for seven days The other forty mice served as control Result only two of the mice treated with penicillin died the rest were cured Of the untreated animals the 'control' of the experiment, thirty five died, only five survived

Thirty days after the treatment the surviving mice that had been cured by penicillin were killed and thoroughly examined There was no sign of infection But when the livers and spleens were tested for the presence of the germ it was found that not all the virus organisms had been destroyed by the drug In a latent form the germ was present in the organs of the mice

These experiments are not conclusive but they indicate that penicillin might be of some help in very large doses when the virus of ornithosis causes infection in man

ABSORPTION OF PENICILLIN AND METHOD OF APPLICATION

The first steps in the investigation of any antibacterial substance are the experiments *in vitro*, in the test tube. They supply important information as to the behavior of the germ in the presence of the drug. These experiments are never conclusive. They are only indicative, suggesting the potentialities of the drug. The experiments *in vivo* on animals, comprise the most essential phase of the investigation. Here the investigators learn whether the drug is capable of preventing or curing specific infections and also gather valuable data regarding the size of the dosage and the best method of treatment.

In this respect, extensive research was done on penicillin. From the beginning of the original investigation it became evident that this substance, when introduced into the system disappears very quickly. It does not remain either in the blood, the intestines or in the organs for any long period of time. It is excreted by the kidneys as if by magic. When given by mouth it is partially destroyed in the intestines and only about 35 per cent actually reaches the blood. From there it is eliminated again through the kidneys. To maintain penicillin treatment in the blood and tissues continuously for a long period of time was the fundamental problem of the investigators. For penicillin is effective against germs only if it is present in the blood or in the infected area continuously for a period of hours or days, depending upon the gravity of the infection.

In order to combat an infection which is general and to destroy the germ which has penetrated into the blood or vital organs, the level of penicillin maintained in the blood should be between 0.03 and 2.0 units per cubic centimeter. That is a very small concentration, indeed, but even such an amount in the blood is attained with difficulty. If 50,000 units of penicillin are injected into the vein, the level of

penicillin in the blood will show a rather high concentration, about 3.0 units per cubic centimeter. However, two hours after the injection the level of penicillin will fall to only 0.06-0.09 unit per cubic centimeter, a concentration which is already below therapeutic effectiveness.

A person suffering from a streptococcus infection, which has spread into the circulation and caused blood infection, should have the level of penicillin maintained continuously at about 0.8 unit per cubic centimeter of blood. How can this be achieved? In cases of grave infection, where penicillin must be maintained at the highest possible level, a method of continuous injections is recommended. Penicillin in solution is given by the drip method, as in some cases saline is administered after an operation. The drug enters the vein or muscle drop by drop continuously for as many hours or even days as is necessary. In order to keep penicillin in the blood on a level of 1.0 unit per cubic centimeter, the total dose of the drug given per day should not be less than 1,000,000 units.

When penicillin is administered by intramuscular injections, as is usual in daily practice, the drug disappears from the blood much more slowly. Thirty minutes after an injection of 100,000 units, the blood would show about 1.5 units per cubic centimeter of blood. But two hours after the injection it will drop to only 0.6-0.8 unit. If the infection is mild, or the germ which caused it highly susceptible to penicillin even such a small amount is sufficient to produce therapeutic effect. Therefore, muscular injections of 100,000 units every three hours might suffice to cure an infection, even if it is generalized. However, for a complete sterilization of the blood from the germs, the injections should not be spaced at longer intervals than three hours. That is the limit for proper treatment. Of course, if the infection is very grave, and the germ is known to be a streptococcus, it might

be advisable to increase the dose of penicillin or administer it every two hours.

Why is penicillin not prescribed in the form of subcutaneous injections? Actually, when penicillin is introduced into the skin instead of the muscle it disappears somewhat more slowly. A relatively high level of penicillin was observed after such injections. However, subcutaneous injections are more painful; perhaps that is the reason doctors avoid them. Some physicians do recommend this type of injection for children and infants.¹³

Many methods have been proposed to prolong the action of penicillin by delaying its absorption. The most effective, however, is the one elaborated by M. J. Romansky and G. E. Rittman, who recommend the suspension of penicillin in peanut oil and beeswax. This form of penicillin injection has received general recognition and is widely administered by the medical profession. Certain oils delay absorption of medicinal substances when injected intramuscularly. The combination of an oil and beeswax seems to produce an even greater delay in absorption. The delay is so pronounced that twenty-four hours after the injection of penicillin in oil and wax the drug is still present in the blood, although on a relatively low level.

Quite an extensive investigation was conducted in order to determine the exact delay in absorption by this method. The figures of the investigators vary considerably, but this method apparently makes possible the reduction of injections by about half. In cases of mild infection, the injections of 100,000 units every eight hours should be sufficient to produce a therapeutic effect. When the infection is grave and the germ is very resistant to the drug, the intervals between the injections may have to be reduced to five or six hours. Many physicians are satisfied with the administration of oil-wax preparations of 300,000 units of penicillin twice per day.

Much attention has been paid to the administration of penicillin by mouth. It is a much simpler and more convenient method. In many instances the injections of penicillin require the presence of a nurse almost continuously. There is no doubt that penicillin taken by mouth gradually gains its way into the blood. But in order to reach a high level much larger doses of the drug must be taken orally. It is estimated that about two thirds of the penicillin is wasted either through the destruction by gastric juices or by the intestinal bacilli. Thus when one takes penicillin by mouth the dose should be about five times larger than one injected intramuscularly. Instead of 300 000 units per day injected into the system more than one million units must be taken by mouth to obtain the same therapeutic effect. In mild forms of infection smaller doses may produce a curative effect. In many instances it is advisable to administer penicillin simultaneously by mouth and by intramuscular injection. The dose depends entirely on the type and gravity of the infection.

TOXICITY OF PENICILLIN

Fleming was the first to observe that penicillin had a low toxicity for animals. Since then it has been firmly established that penicillin in the medium of calcium sodium or potassium salts is remarkably low in toxicity for many animals. When the drug is injected into the veins or muscles of the animal in a dose sufficient to arrest infection no harmful effect is observed. However much larger doses might kill the animal. The amount must be about sixty four times that of the therapeutic dose in order to kill mice by subcutaneous injection. Penicillin given to animals by mouth is completely harmless. Among various preparations of the drug the least toxic for animals and men is the sodium salt of penicillin.

Generally speaking when penicillin is given to man by

mouth it produces no harmful effect at all. There is no nausea, vomiting, or diarrhea, with very rare exceptions. Intravenous injections, however, often cause some general reactions. Flushing of the face, headache, slight fever, and pain in the muscles may occur. One opinion expressed was that these reactions were caused by some impurity in the drug. Such an assumption may be sound. For as the method of purification of the drug has advanced and the preparations of penicillin on the market have improved in quality, the gravity of these reactions has lessened somewhat.

The toxicity of a drug must be considered in terms of the damage it may cause to the red and white blood cells, the bone marrow, and the vital organs of the body. Precisely in this respect the low toxicity of penicillin is valuable. As Dr. W. J. Morginson remarks, in his review on the toxic reactions in penicillin therapy, "Apparently there is no disturbance of the peripheral blood or hemopoietic system, and penicillin can be used in the presence of pronounced anemia, leukopenia, and agranulocytosis." Doses as large as 400,000 units in experimental animals produced no injury to kidneys, liver, bone marrow, or brain. Hence a dose of several million units administered to an adult man would not harm his vital organs.

However, clinical evidence indicates that penicillin possesses definite allergenic properties and that it may cause allergic reactions. Sensitivity to the drug may manifest itself in an immediate response, or as a delayed reaction. The first type of reaction occurs, as a rule, in persons with a known history of penicillin sensitivity, who have acquired it from penicillin therapy. There may, however, be persons who are naturally sensitive to penicillin, although apparently their number is very small. M. N. Kolodny and E. Danhoff suggest that some persons may have a natural allergy to penicillin.¹⁴

Delayed reactions of sensitivity may appear as the result

of repeated injections of penicillin into the skin or may be due to the local application of the drug in ointments and pastes. Allergic symptoms following penicillin therapy are usually mild and of a temporary nature although in persons who show a strong allergy to penicillin the irritation may be of considerable severity. The most common reaction from penicillin is urticaria and dermatitis. It is estimated that it occurs in about 1 to 2 per cent of treated persons. Some persons experience such severe skin irritation that the treatment must be stopped.

What can be done in such cases? The penicillin-sensitive person may be desensitized according to some investigators.

Dr S M Peck and his associates at the Mount Sinai Hospital in New York reported an interesting case of a patient, allergic to penicillin whom they successfully desensitized.¹⁵

A white man of sixty three with acute respiratory distress was admitted to the hospital. He was given injections of penicillin for four consecutive days. Altogether he had received 800,000 units of the drug when he developed an acute dermatitis. The skin eruption appeared first on his hands, feet and groin then spread rapidly to involve his entire body. His skin showed a scarlatin like type of eruption. His face was swollen and he felt quite miserable. The penicillin treatment was arrested. Gradually the eruptions disappeared although the skin on the hands remained in a state of irritation for almost a month. Because the patient's condition required penicillin the doctors began to give him very small doses three times a week. With every injection the dose was slightly augmented. After three weeks the patient was "desensitized" and was able to tolerate relatively large doses of penicillin.

When syphilitic patients are treated with penicillin they

often manifest therapeutic shock (Herxheimer reaction) It usually occurs on the first or second day of treatment The lesions appear to be accentuated and the symptoms of the disease aggravated Drs Mahoney and Arnold have observed such a reaction in 86 per cent of the syphilis cases treated with penicillin Similar figures are given by other investigators This reaction is interpreted as the favorable response of the organism to penicillin These symptoms however can be prevented by the administration of smaller doses during the first few days

When penicillin is applied to the central nervous system it produces a definitely irritating effect According to C A Neymann G Heilbrunn and G P Youmans penicillin is toxic when it comes into direct contact with the surface of the brain It may produce headache muscular twitching and even convulsions These symptoms are considerably reduced after the dosage of the drug is diminished A large dose of penicillin does produce an irritating rather than strictly speaking a toxic effect on the nervous system When 5 000 units are injected into the brain cavity no reaction occurs But when the dose is 20 000 units there may be a slightly irritating effect Many patients however have received from 20 000 to 100 000 units injected intrathetically without reactions Nevertheless doses higher than 30 000 units should be used with great caution in direct application to the brain surface

COMBINED ANTIMICROBIAL ACTION OF SULFA DRUGS AND PENICILLIN

There are bacteria which show considerable resistance both to sulfa compounds and to penicillin The question arose whether these two drugs given at the same time would not be more effective in certain resistant types of infections Do these drugs act synergistically, that is do they combine

forces? In this regard Dr C M Carpenter has made an interesting observation. While gonococci may become readily resistant to sulfadiazine in the test tube they are unable to form such a resistance when penicillin is added to the medium containing sulfadiazine. Some other investigators (Ungar, Chain and Duthrie) believe that these two drugs might exercise their action on the bacteria in a synergistic manner. But on the other hand Hobby and Dawson were not able to observe in vitro such synergistic action of sulfadiazole and penicillin against staphylococci and streptococci.¹⁶ This question still remains open for further discussion and investigation. But clinical evidence seems to accumulate which favors such combined treatments for many cases of stubborn and dangerous infection. This evidence indicates that in a number of infectious diseases the effectiveness of penicillin is enhanced by sulfadiazine. Moreover the sulfa drugs are not the only ones which may increase the antibacterial activity of penicillin; some substances like amino acids, may enhance its effectiveness equally well.¹⁷

Several fundamental facts are now firmly established about penicillin as an antimicrobial agent.

It is effective against most but not all Gram positive organisms.

It shows little if any activity against most Gram negative bacteria.

With very few exceptions it is ineffective against viruses.

Among the species of bacteria which are as a rule highly susceptible to penicillin are some strains which manifest a natural resistance toward the drug. A typical bacterium in this respect is *Staphylococcus aureus*.

Moreover for some unknown reason penicillin is not capable of destroying all the germs exposed to its action. A very small percentage of the bacteria evade the destructive action of penicillin and remain alive. These survivors may

gradually acquire a resistance against the drug. For this reason it is recommended that penicillin treatment should be started with as large a dose as possible, in order to forestall the growth of a new generation of resistant germs.

Various bacteria possess different degrees of susceptibility toward penicillin and the dosage of the drug for treatment is figured according to the type of germ causing the infection.

Penicillin is a drug of very low toxicity for man and animals.

It does not harm the blood-forming system and does not dissolve the blood cells. Therefore it can be administered safely to anyone who may be suffering from anemia.

There is, however, a small percentage of people who seem to be naturally allergic to the drug and who manifest symptoms typical of allergic reactions. It is estimated that one out of one hundred persons is allergic to penicillin. These allergic reactions are neither grave nor dangerous but might become quite acute. In such a case the treatment should be interrupted promptly.

There is some evidence suggesting that the addition of sulfadiazine may enhance the effectiveness of penicillin against some germs. This evidence is much more of a clinical nature than of experimental origin.

8

Penicillin vs. Infectious Diseases

THE FIRST REPORT on the treatment of human beings with penicillin was published by Dr Dawson and his associates in 1941.¹ Since the commercial drug was not at that time available the Columbia University workers prepared their own penicillin. The results were encouraging but not conclusive.

In 1943, after small amounts of penicillin were distributed among a few hospitals and a number of various infectious diseases were treated under strict control, a report covering 500 cases was published by Dr Keefer and his associates.² This report outlined the potentialities and the limitations of the drug.

Many cases treated with penicillin were failures. Dr Keefer frankly admitted that many of these patients were inadequately treated with respect to the total amount of penicillin they received and to the total duration of treatment. A number of the patients were treated early in the course of studies when very small amounts of material were available and when little was known about the dosage.³ Nevertheless, the report was enthusiastic. The investigators were impressed by the absence of toxic reactions. 'One of the remarkable features of penicillin,' wrote Dr Keefer, 'is its

low toxicity' From that time the clinical investigations proceeded on an ever enlarging scale Today, the whole picture of what penicillin can do and what it cannot do has been more or less resolved.

When germs succeed in penetrating the first line of defense of the body—either the skin or the mucous membrane—infection results This infection may remain local if the defense forces of the organism, the second line of defense, are strong enough to localize the invaders. A boil is a local infection, so is an infected tooth But if the germs are virulent and the resistance of the organism is weak, they may penetrate into the blood stream When bacteria are present in the blood stream *septicemia*, or blood infection, takes place *Septicemia* means an infection of the blood which causes high fever and other dangerous symptoms Gradually, if the germ invades other vital parts of the organism, the death of the patient may occur

BLOOD INFECTION (SEPTICEMIA)

Blood infection is a baffling condition with which doctors have hitherto been powerless to cope Based on the first clinical cases treated with penicillin the discovery of this drug has given new hope to the medical profession In the past only twelve to fifteen out of one hundred patients had a chance to recover from blood infections When sulfa drugs were introduced the ratio went up to 35-38 per cent.³ With penicillin the chances are much brighter It is estimated that out of five persons affected four, or 80 per cent, have an excellent chance to recover their health completely Although blood infection may be caused by many germs in medical practice we deal mostly with infections caused by staphylococci streptococci and less frequently, by meningococci In septicemia caused by streptococci penicillin is slightly less effective than in the case of staphylococcus and, consequently,

much larger doses must be given.⁴ Whatever germ invades the blood, it is essential to begin the treatment with very large doses of penicillin intravenously and intramuscularly. The most effective method seems to be continuous injections.

Many dramatic stories have been told of the successful treatment of blood infection.

There was the case of a sixteen year-old college student, the only daughter of a prominent New York physician. Her illness developed with distressing swiftness. Five days before she entered the hospital she had been in perfect health. She began to complain of soreness on the left side of her nose. Redness and swelling developed and gradually extended over the entire face. Both of her eyelids became so swollen that she could no longer see. A rapid pulse and a fever of 104° indicated the danger of her condition. A blood examination confirmed the diagnosis that her father had already made: septicemia, blood infection, caused by the blood-destroying streptococcus. Days of treatment brought no sign of improvement, but a decline in her condition. The girl was dying.

Penicillin, the only remaining hope, was practically unobtainable at that time. When her father finally obtained a release on one million units, the girl was already in a semi-comatose state. The first day brought no apparent change. All hope of saving her had been abandoned. Suddenly, her temperature began to drop and she regained consciousness. A few days later her blood was free of the germs. She could see again, and could even take a few spoonfuls of nourishment. Small wonder that her father has since been one of the most ardent adherents of this new drug.

Another case concerned a fifteen year-old high school boy. He hurt his leg while working in the garden and neglected the infection that developed. At first only the flesh was involved, but gradually the inflammation reached the bone. A condition known as *osteomyelitis* (infection of the bone

tissue) resulted. The germ finally found its way into the blood stream, and an exhausting fever set in. When the blood was examined bacteriologically, the pus-forming *Staphylococcus aureus* was identified. Gradually the lad's condition became grave, almost hopeless. Sulfa drugs brought no relief.

The boy was completely emaciated and on the verge of death, when at last it was decided to give him penicillin, which, at that time, was still difficult to obtain. His recovery was very slow. Only after seven days on each of which more than 200,000 units were injected, were there any signs of improvement. Nevertheless, after fifteen days of continuous treatment he was out of danger and in a satisfactory condition, although it took many weeks before he was restored to normal health. That was one of the closest calls on record for penicillin, for hours, or even minutes, might have meant the difference between life and death.

A similar, but not quite identical, case of blood infection was described and reported by Dr. Charles O. Ericksen. The initiator of infection was also staphylococcus, but of a slightly different variety, *Staphylococcus albus*. This germ is even more of an opportunist. It can be found everywhere, even on an apparently clean skin. This germ, too, caused a blood infection that might have been fatal had it not been for penicillin.

The patient was a healthy young farmer, twenty-nine years old. In September, 1943, his throat became sore and he thought he had contracted flu. The illness persisted, he had chills and fever, complained of pain in his muscles and bones and of a stubborn headache. The pain became particularly severe in his right hip. In three weeks he had lost about twenty pounds and was so weak that he could hardly move.

Admitted to the hospital, he was examined, and his blood

showed that the common germ *Staphylococcus albus* had invaded his system and caused the inflammation of the soft parts of the bones (osteomyelitis). The patient was given large doses of sulfa drugs which did not help him at all. His weakness was increasing dangerously. He was given a blood transfusion which bolstered him temporarily but did not do him any lasting good.

He had now been ill for seven weeks and was steadily losing ground. His condition was critical if not altogether hopeless. Then penicillin treatments were ordered. For about ten days he received a daily dose injected intravenously. On the day following the first injections the patient's temperature dropped to normal and he felt better. At the end of ten days no germs could be found in his blood. A week later the young farmer was allowed out of bed, he felt well and was eager to get home. The only evidence of his illness was a slight pain in the right hip.

Until now the blood infection which sometimes follows abortion was considered almost incurable. There was but a slight chance of recovery and there was little the doctor could do to help the patient. One case reported recently by a New York doctor seemed utterly hopeless and yet was cured by penicillin.

The patient was a healthy young Italian woman married and the mother of two small children. She could not afford a third child. Yet she had become pregnant. What does a woman with a meager income do in such a case? She had an illegal abortion.

A few days later her temperature went up. Her whole body ached. She went to a hospital and soon after admission suffered a severe hemorrhage. The next day her temperature mounted to 104-105° and remained there in spite of heavy doses of sulfa drugs. When her blood was examined *Staphylococcus aureus* was found. The germ had invaded not

only her blood, but her lungs as well. The patient developed a grave pneumonia in both lungs. As usually happens in the case of abortion, when the infection spreads widely through the system, the inner lining of the abdomen is also affected by the germ, and peritonitis sets in. The woman's chances of survival were poor indeed, with the germs circulating in her blood, the lungs affected, and the inflammation of the abdominal cavity. She was already in a semiconscious state, near death, when penicillin was given to her for the first time.

On the first day 100,000 units of penicillin were administered every three hours. The next day the patient received a dose of 200,000 units every three hours. The third day her temperature dropped to normal. But the woman was still not rational, and was unable to take any food. However, two days later, having received penicillin injections every three hours, night and day, her condition improved, and she was able to take fluid food. Her lungs began to clear up, and the swelling of the abdomen decreased. She improved from day to day, and her blood remained free of germs. Soon after, she was well enough to go to a convalescent home.

Dr. Carl G. Harford and his associates of Washington University Medical School, St. Louis, reported a typical case of septicemia:

A man of fifty-four was apparently in good health until four days before he was admitted to the hospital. On the day before entry he began to have chills and fever, and on the following day he complained of severe chest pains. When first examined at the hospital the whole right side of his face was swollen. A large dose of sulfamerazine had no effect whatsoever, so penicillin treatment was started. During the first twelve hours in the hospital his condition rapidly became worse. Examination revealed an infection of both lungs. Blood cultures showed *Staphylococcus aureus*. He re-

ceived penicillin continuously for thirteen days intramuscularly, and penicillin also was introduced into his pleural cavity. Recovery was prompt. In this case the infection had begun with the formation of a furuncle of the nose. The germs spread into the blood stream, invaded the lungs, and caused double pneumonia and pleurisy.

The successful treatment of blood infection depends on the proper administration of large doses of penicillin as soon as the signs of septicemia are recognized. In some instances a combined treatment of penicillin and sulfadiazine seems to give the most encouraging results.

INFECTION OF THE LUNGS (PNEUMONIA)

In the year 1880 Louis Pasteur discovered a germ that later became known as the pneumococcus. At that time he was not sure whether this germ was the one that caused pneumonia, the grave disease in which the lungs are infected and badly inflamed, and from which men often die.

Although various forms of germs are capable of invading the lungs and inducing pneumonia, it is the pneumococcus that has 'specialized' in this field. There are many types of pneumococcus, some less dangerous to man, others very aggressive and virulent. Altogether there are about thirty-two strains of pneumococcus divided into several groups, but Type I and Type II each account for about one third of all cases. The most dangerous pneumococcus is Type III.

Pneumonia is a major problem facing every general practitioner. In the early spring and late fall, when colds and influenza ravage the population of large cities, the doctors are confronted with large numbers of pneumonia cases. In every instance, but particularly in cases where the patient is no longer young, there is always the danger of some serious complication and of possible death. Medical men have been seeking a long time for a drug which would prove reliable

and effective in arresting the development of this disease, and in preventing the complications which so often accompany it.

Penicillin has almost solved this problem. The present figures indicate that pneumonia incited by any type of pneumococcus can be arrested in 93 per cent of cases. Moreover, the dosage of penicillin need not be very large. In most cases not complicated by other infections the illness may terminate in complete recovery within three or four days. The total amount of penicillin required for the successful treatment of pneumonia is as a rule, not more than 500 000 units.

As early as 1943 Dr. Keefer stated: "It is plain from the report cases that penicillin is a potent weapon in the treatment of pneumococci pneumonia and many patients have recovered on less than 100 000 units given over a period of two or three days. Others have required more penicillin. The recent clinical data on this subject fully corroborates Dr. Keefer's report. At present, however, the total dose of penicillin given to a patient with pneumonia is larger than the ones administered when the drug was scarce. When there are complications in the pneumonia this is imperative."

An instructive case along these lines was recently reported. A sixteen-year-old girl had suffered frequent headaches and pain in her right ear since childhood. Three weeks before hospitalization she noticed drainage from her right ear, and complained of pain in and behind the ear. Twenty hours before her admission to the hospital she was seized with a severe headache and fainted. Although she promptly regained consciousness the headache continued and gradually she became delirious. Examination revealed pneumonia, complicated by meningitis and otitis. For six consecutive days she was given 200 000 units of penicillin daily. This treatment

brought her complete recovery. Ten years ago such a case would probably have been considered hopeless.

The pneumococcus is not the only germ causing pneumonia. Our opportunists staphylococci and streptococci, sometimes also take advantage of the low resistance of the organism to invade the lungs and to cause as grave an infection as do the pneumococci. Unfortunately penicillin is somewhat less effective in such cases and the chances of recovery with the help of penicillin are slightly smaller than in the case of the pneumococcus. The percentage of recovery for staphylococcal pneumonia treated with penicillin is about 85 to 87 per cent while streptococcal pneumonia is even more resistant and only 60 to 65 per cent have a chance to be cured.

Two English doctors T. I. Bennet and T. Parks described several cases which they had been able to cure with penicillin. The climate of England favors diseases of the respiratory system. Influenza and common colds are very frequent. As often as not, a simple cold or flu is complicated by a lung infection. In fact all the patients treated by these English doctors had started with a mild case of influenza which had turned into dangerous broncho-pneumonia.

One of their patients was a man of fine physique and in good health in his early forties who lived in a small town. He came to the country hospital for some minor ailment and caught a cold while staying there. By the fourth day he had a temperature of 101° and signs of bronchitis appeared. On the fifth day the bronchitis turned into broncho-pneumonia. His sputum was full of *Staphylococcus aureus*. Intensive treatment with sulfa drugs lasting several days brought no improvement. When he was delirious and at the point of death the doctors decided to give him penicillin. He was sent to Middlesex Hospital where he was subjected to ener-

getic penicillin therapy. He recovered after ten days of treatment.

Penicillin is of little, if any, help in the case of virus pneumonia (Reimann's pneumonia) as well as in the pneumonia caused by the virus of parrot fever (atypical pneumonia)

A middle-aged woman writer was admitted to the hospital in grave condition. At home her physician had diagnosed her ailment as pneumonia. She received the usual treatment, first with sulfa drugs and, when she did not improve, with penicillin. At first she seemed to respond to penicillin but a few days later she lost ground. She was suffering from an atypical pneumonia. Her sputum was examined. A test made on mice left no doubt that she was affected with 'avian psittacosis'.

Both psittacosis, or parrot fever, and the similar infection transmitted by pigeons, usually begin with pneumonia like symptoms, high fever and inflammation of the lungs but the infection soon spreads to other organs of the body. Liver and spleen may be affected and paralysis of the legs and arms is often present. In most cases the infection is fatal and ends in the death of the patient. No effective treatment against it is known. In spite of intensive treatment with penicillin the woman died.

At one time there was hope that penicillin might be able to combat this dangerous infection. As was noted earlier, the experiments on animals gave reasons for such hope, but the clinical evidence was not so encouraging.⁶

Penicillin may be of help in asthma caused by infection of the bronchial tubes and chronic bronchitis. The drug is usually introduced by inhalation and in some cases highly satisfactory results have been reported.⁷

When the lung is operated on for some reason, penicillin

renders considerable protection by preventing postoperative infection ⁸

All in all penicillin is the most powerful drug in the treatment of pneumonia. Because of its harmlessness it can be given to older people without any danger of ill effect.

INFECTION OF THE BRAIN COVER (MENINGITIS)

As soon as penicillin was discovered it was hoped that the drug would provide a cure for meningitis. However, the first cases treated with penicillin were distressing failures. Theoretically penicillin should have been effective because the meningococcus germ, as was proved in the test tube, was very susceptible to the drug. The cause of the failure was soon uncovered.

Dr. Charles Rammelkamp of the Evans Memorial Hospital in Boston, together with Dr. Keefer, solved this medical puzzle. They found that the drug, when given to the patient intravenously, only partially reached the brain. Only a small quantity of penicillin was found in the spinal fluid of persons who had received large amounts of the drug. Something was preventing the penetration of penicillin through the barrier that divides the brain and spinal cord from the rest of the human body. The penicillin remained as potent as ever if only it could reach its destination. To bring the drug to the infected area, the Boston scientists introduced penicillin directly into the spinal-cord cavity. At first they were very cautious and gave the patient a relatively small dose, not more than 10,000 units. The results were gratifying. The patient was saved.

Using the method suggested by Rammelkamp and Keefer, other practitioners have administered penicillin to meningitis patients. Dr. Albert Evans tells of a case in which he saved the life of a man stricken with meningitis when there seemed to be no hope left.

The patient was a serviceman thirty five years old His swamp glider had overturned and the propeller had struck him on the head He was admitted to his station hospital in an unconscious state The wound was debrided After a few weeks in the hospital he returned to his quarters Soon he became very ill experiencing nausea headache and all the other signs of inflammation of the brain cover His spinal fluid revealed germs of the pneumococcus type Penicillin was injected simultaneously by intravenous and intramuscular methods and directly into the spinal-cord cavity Altogether the patient was given 2 088 000 units

His improvement said his doctor was progressive and he made an eventual recovery Survival would not have occurred with the types of therapy in practice prior to the advent of penicillin

At present the mortality from meningitis caused by the meningococcus is reduced to about 10 per cent But the therapy should follow the pattern advocated by Rammelkamp and Keefer namely injections into the veins or into the muscles simultaneously with injections into the spinal cavity Since meningococcus is also susceptible to sulfa drugs a combined treatment with sulfadiazine is often used with excellent results⁹

Meningitis caused by pneumococcus is much more resistant to treatment with penicillin and the mortality from this condition is reduced only to about 60 per cent¹⁰ The meningitis caused by staphylococcus responds quite satisfactorily to treatment with penicillin and recovery may be expected in about 8₇ per cent of cases¹¹

All the clinical evidence concerning the treatment of meningitis with penicillin alone or in combination with some sulfa compounds indicates that the disease once regarded fatal is gradually being brought under control It still claims

these persons become free of the germs and cannot endanger those with whom they come in contact. Dr Hirsh and his associates recommend rather large doses of penicillin to achieve a complete recovery. The patients were given 125 000 units of penicillin every three hours for not less than five or six days.

Dr Archibald L. Noyne and Rowine Hayes Brown of Chicago corroborated these findings. They treated successfully one hundred and sixteen patients for scarlet fever. Each patient received about 500 000 units altogether, a much smaller dose than that administered by Dr Hirsh and his associates. Dr Noyne and associates concluded that 'penicillin is superior to sulfonamide drugs as a therapeutic measure for scarlet fever.

INFECTION OF THE HEART (SUBACUTE ENDOCARDITIS)

Among the opportunists who live with us and on us there is a coccus which forms a green colony; this germ is *Streptococcus viridans*. It is common when a mild infection occurs. When a tooth abscess develops in all probability it is the green-colony germs which are to blame. Many a sinus infection is due to the activity of this mild appearing opportunist. In inflammation of the appendix or gall bladder one must suspect the same germ. But in some fortunately rare conditions the green-colony germs succeed in penetrating into the blood stream and reach the valves of the heart. As a rule they attack weak hearts—often rheumatic hearts. There the germs grow, forming vegetations which consist of the masses of streptococci encamped in the inflamed tissues of the heart valves. This dangerous condition, which until very recently resisted all treatment is called *subacute endocarditis*. Although other germs can and do cause this heart infection the green colony germ is responsible for this distressing illness in the majority of cases (96 per cent).



DR. SELMAN WAKSMAN, DISCOVERER OF STREPTOMYCIN

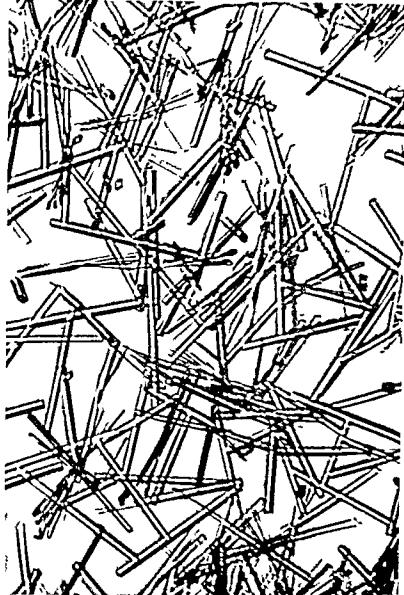


Photo from Merck & Co. Inc.

CRYSTALLINE STREPTOMYCIN UNDER THE MICROSCOPE

The peculiar characteristic of this disease is that it may affect young or old, healthy or ailing persons, and its onset may originate from a trivial ailment like an infected tooth or a head cold. When sulfa drugs were introduced great hopes were placed upon them, but they failed to fulfill expectations. With the discovery of penicillin new hopes arose, but the first clinical observations were not very promising. Five years ago Dr. Keefer frankly admitted that "with regard to subacute endocarditis the picture is confused." However, with an improved technique the effectiveness of penicillin in this disease has been considerably increased.

Why did penicillin fail at first in the treatment of this condition? Is this drug not effective against the germ that causes infection of the heart valves? The drug is known to be active against *Streptococcus viridans* and is highly effective in blood infections. And yet, apparently, it has been ineffective against the same germs when they are firmly encamped inside the heart.

Gradually, as experimental and clinical evidence accumulated, three factors emerged which accounted for the failures of penicillin therapy in the first attempts. It was learned that a peculiar physiochemical condition is present in the heart tissue which handicaps to some extent the antimicrobial activity of the drug. Another factor of major importance is the dosage of penicillin used. The total dose, even as much as was recommended in blood infections, was still not sufficient in subacute endocarditis. The final factor, which may account in part for the failure of the penicillin therapy, was the resistance of the germ.

What conditions are present in the heart valves which might interfere with the activity of penicillin? The valves of the heart are rich in red cells. In the event of inflammation an excess of a fibrinous substance may be found around the encamped germs. Is it possible that this fibrin-like barrier

forms some sort of wall around our enemies and that this wall obstructs the penetration of penicillin and impedes the entrance of the white cells?

Dr Louis Katz and Dr Stephen Elek held that this was precisely the case and developed an ingenious new therapy. To prevent the formation of the fibrin wall around the infected area in the heart they tried to prevent the formation of blood clots the point-of-departure for the accumulation of the fibrin. To make the blood flow more freely they used heparin a substance which is naturally present in the liver and which retards the clotting of blood.

Dr L. Loewe and his associates have used penicillin and heparin on patients affected with inflammation of the heart valves. They have frequently though not always been successful.

Altogether they have treated sixty two patients suffering from endocarditis and have had forty six recoveries. In most cases the offending organism was a *Streptococcus viridans*. From 150 000 to 200 000 units of penicillin were given daily the total ranged from 1 400 000 to 7 800 000 units. The results of this combined penicillin and heparin therapy seem very encouraging. Although Dr Loewe and his associates are restrained in their conclusions they say that the immediate effects [of this therapy] suggest uniformly successful sterilization of the blood and relief of clinical manifestation. So far forty six patients are alive and in good condition. However the investigators are right to conclude their report with "further observation will be required to determine the permanence of results".

Some of the cases they describe are particularly striking for example that of the girl L. Z. aged seven. She was admitted to the hospital because of chills and fever of ten weeks duration. Three years before admission she had a bout of unexplained fever which lasted eight weeks yet she

remained perfectly well thereafter until her present illness developed. Her temperature ran as high as 103.8° with a fast pulse of 132. Examination revealed bacterial endocarditis caused by pneumococcus. She was first treated with large doses of sulfa drugs without any apparent improvement. Then it was decided to submit her to the penicillin-heparin therapy. During the first three days she was given 223,000 Oxford units of penicillin and a hundred or two hundred milligrams of heparin every second day. Although her general condition was good, the temperature persisted and the penicillin-heparin treatment was continued without interruption for two weeks more (for a total of 867,920 units of penicillin and 1,200 milligrams of heparin). Her blood cultures, taken repeatedly during this time, were sterile, showing no sign of the germ. Before long she was discharged for further convalescence at home. "Since then she has been examined periodically, her temperature remains normal, and the blood cultures negative. She is now attending school regularly."

Another case concerned a man of thirty-one. When he was a child of six, he had an attack of rheumatic fever from which, however, he apparently recuperated. His health was generally satisfactory, except for his teeth, several of which were infected; he seemed to have paid little attention to the condition of his mouth. Three weeks prior to his admission to the hospital he felt ill. The toes of his right foot became painful and red. His temperature rose slightly. When his blood was examined, there was no doubt that the green-colony germ had invaded and attacked the valves of the heart. The diagnosis was obvious—endocarditis. After he was treated with heavy doses of sulfa drugs without appreciable improvement, the penicillin-heparin therapy was undertaken. He was given large doses of penicillin, altogether 1,400,000 units during two weeks, and made a dramatic re-

covery The germs disappeared from his blood and his temperature returned to normal In fact he gained fourteen pounds during the six weeks he was under treatment and observation When his infected teeth were removed the germ which had caused his heart trouble was found to be present His general condition at discharge was most satisfactory the doctors reported ¹²

Subacute bacterial endocarditis declared Dr Charles K. Friedberg of Mount Sinai Hospital is now the most common form of heart disease which can be cured ¹³ Reviewing 148 cases of this disease treated since the advent of penicillin in 1944 and up to June 1949 Dr Friedberg stated that penicillin is the most reliable antibiotic for this disease The duration of treatment has generally been a minimum of five weeks There is a continued trend towards the administration of larger doses Although a dose of 500 000 units daily is often adequate for a high percentage of cases especially those caused by nonhemolytic streptococcus it is wiser to recommend 1 000 000 units of penicillin as the minimum daily requirement When the disease is caused by a less sensitive microorganism dosages as high as 5 000 000 units or more per day are being used At present up to 70 per cent of cases of subacute endocarditis are being cured by penicillin therapy If we compare these figures with 1 2 per cent of recovery in the pre sulfonamide era or with 4 9 per cent of the sulfonamide era one may see the enormous progress achieved in the treatment of this deadly disease This disease requires a long continuous therapy in order to sterilize the heart tissue of the germs Direct killing of all bacteria is important in curing bacterial endocarditis remarks Dr Thomas H Hunter of Washington University And since penicillin is bactericidal and affects the germ directly it should be given a preference over all other antibiotics

Thus penicillin therapy has opened a new and very hopeful chapter in the treatment of this disease which only a few

years ago was considered by the medical profession as hopeless

GNORRHEA

Only a few months after the British Drs Florey and Heatley arrived at Peoria, doctors at the Mayo Clinic undertook the treatment of gonorrhea with penicillin. They had at their disposal only a very small amount of the drug prepared in their own laboratory. No one had yet tried penicillin on this disease, not even the British workers. Dr Wallace Herring and his associates at the Mayo Clinic were the first to discover the extraordinary efficiency of penicillin in gonorrheal infection, as evinced in the first two cases treated with this drug.

A man admitted to the clinic had contracted a gonococcal infection a few weeks earlier. An inflammation set in involving the prostate gland. The infection was acute. The patient was treated with an adequate course of sulfa drugs but since the benefit of this therapy was only slight and temporary, the sulfas were repeated. He received an even greater amount of the drug, but the inflammation seemed to become aggravated. Finally, the doctors resorted to penicillin. The patient was given repeated injections of the new drug—about 30,000 Oxford units during the first twenty-four hours. To the amazement of the doctors, five hours after the first dose was given, the patient felt better. When ten hours had elapsed, no more germs could be found. They had all vanished, destroyed by penicillin. Twenty-four hours later all symptoms of his condition had disappeared. The man was completely cured and all his subsequent tests were negative.

Thus a disease which would ordinarily take weeks or months, to cure, and which is frequently followed by complications and physical distress, not to mention the psychological anxiety of the affected person, was cured by penicillin within a few short days.

Even more striking was the second case treated at the Mayo Clinic. The patient was a young man of twenty nine. For five weeks he had been suffering from a gonorrheal infection with a great deal of abdominal pain. He ran a fever of 100° 101° . Various sulfa compounds were tried on him with unsatisfactory results. After a slight improvement the infection would return with renewed vigor. The doctors were discouraged and finally resorted to penicillin. The result of the treatment was miraculous. Only five and one-half hours after the new drug was first given the patient announced that his pain had completely disappeared—perhaps a psychological reaction. Yet within forty hours no trace of gonorrhea germs could be detected. They had simply vanished. The patient's temperature returned to normal and by all medical standards he was now a healthy man. However to make certain that the infection would not return he was kept in the clinic for an additional two weeks. The cure was permanent.

These two instances are not exceptional. Other cases of gonorrhea even of the most stubborn and resistant form respond to penicillin in the shortest possible time. Of 129 cases treated by Dr. Mahoney at the United States Marine Hospital on Staten Island only four were not completely cured.

In their article "Management of the Venereal Diseases in the Army" Colonel Thomas B. Turner and Major Thomas H. Sternberg reported the results of the treatment of gonorrhea with penicillin. More than 95 per cent of servicemen receiving a total dosage of 80,000 units were cured.

In those patients who responded cure was effected with extraordinary rapidity, subjective and objective improvement occurring in most cases within twenty-four hours after the beginning of treatment. This remarkable drug bids fair to reduce gonorrhea to the status of an inconsequential infection.

There are two striking facts about penicillin's effect on gonorrhea. Its action is very rapid, and it is effective in relatively small doses. Even a dose of 70 000 units or less may clear the system of this germ, a dose of 100 000 units as Commander Harry Oard pointed out, exercises almost complete effectiveness.

Dr Philip Miller of the University of Chicago has made an intensive investigation of the rapidity with which the germs disappear when penicillin is given. He injected penicillin and examined the smear for germs every hour. In some instances one hour after the first injection of 15 000 units was made no more germs could be found in the smear. On an average in three and one half hours all the germs were gone nor did they recur. Patients were completely cured after they had received 60 000 units of penicillin given in the same day during a five to eight hour interval.

Is penicillin as effective in the treatment of women as in the treatment of men? We learn from Dr Alfred Cohn and his associates that women affected with gonorrheal infection have been treated with remarkable results. The same dose effective for a man will suffice to cure a woman. This therapy may be completed within a period of six hours. Two to five injections of penicillin given at an interval of two or three hours, is all that the patient needs to receive. No pain or other bad effects due to the administration of penicillin were observed.

To date about 20 000 cases of gonorrhea treated with penicillin have been reported in the medical press. Statistics show that the percentage of recovery for men is about 94 per cent and for women slightly lower, about 93.1. Gonorrheal prostatitis (infection of the prostate) and gonorrheal salpingitis (acute infection of the ovaries) respond quite satisfactorily to penicillin therapy.

However, individual investigators give figures which vary considerably. Dr R. Koch, of the San Francisco Department of Health, and his associates, stress the fact that careful and repeated bacteriological examinations have given them much higher figures of failure than the ones reported by other investigators. According to their findings, out of 485 gonorrhea patients treated with an initial course of 200,000 units of penicillin, sixty-eight, or 14 per cent, were not cured¹¹

In the treatment of gonorrhea it is imperative that the level of penicillin in the blood be maintained as high as possible during the first eight to ten hours. Thus many investigators suggest that the best method of treating this infection is to give the patient a dose of 50,000 units every two hours (total amount of penicillin 300,000 units). Even more satisfactory results were obtained when penicillin was given in peanut oil and beeswax. A single dose of 300,000 units was sufficient to bring recovery in 91 per cent of the treated cases. According to Dr H. Welch and his associates penicillin X is even more effective than penicillin G. Penicillin X succeeded in curing 94 per cent of their patients.

Although penicillin was proven effective in acute infection of the prostate and the ovaries, it is not so effective when the infections are chronic. And if a woman has a salpingitis of long duration, surgical intervention may still be necessary.

Gonorrhea may be prevented by a single pill of penicillin containing 100,000 units, Dr Harry Engle of the National Institutes of Public Health, United States Public Health Service, recently stated.

The test was made on Navy personnel. The penicillin tablets were given, one tablet per man, to about two hundred men as they returned from shore liberty. This test continued for a sixteen week period. Each time a man from this "experimental group" returned from shore leave he would receive a tablet. In this group only five men contracted gonor

rhea In the 'control' group which did not receive tablets of penicillin, but were given placebo tablets containing nothing but sugar, forty three cases of gonorrhea developed

A few months later the same experiment was repeated but now the men of the first penicillin group received a tablet double the strength of the earlier experimental dose Only one man became infected with gonorrhea When questioned by the doctors he admitted that he had not taken the penicillin pill at all The doctors who conducted the test believe that by this simple preventive method gonorrheal infection can be reduced considerably among men in the services

Gonococci are among the most vicious and virulent germs known The effects of this disease are very serious indeed both for the individual and for society It is the prime cause of sterility In women, chronic gonorrhea is responsible for a long list of diseases many of which require surgical treatment Because of the great frequency of these infections their tendency to become chronic and the grave effect on the individual this disease was considered fully as important a public health problem as the much feared syphilis With the use of penicillin, however, the danger of many of the complications which follow gonorrheal infections will be greatly reduced Gonorrhea itself may, under combined penicillin and sulfa drug therapy, be reduced to the status of an inconsequential disease

SYPHILIS

Drs Mahoney, Arnold, and Harris undertook the first treatment of syphilis with penicillin¹⁵ The infection was fresh—the young sailor had contracted the disease only a few weeks before The examination revealed that the syphilitic ulcer was full of spirochetes and that they were also present in the blood Every four hours for eight consecutive days the patient received 25 000 units of penicillin, altogether 1,200 000 units

were given by intramuscular injection. At the end of the first seven hours of treatment the spirochetes could no longer be found either in the blood or in the ulcer. Blood tests which had been strongly positive for syphilis at the start of the treatment became negative by the fifteenth day.

One hundred days after the treatment had been stopped the blood tests were repeated. No trace of the infection could be detected by the most thorough tests known for this condition. Four months after the sailor had received his first injection of penicillin he was completely cured. Several other patients with recent infections were treated with penicillin and were cured in the same spectacular manner.

Despite the remarkable results Dr. Mahoney had a word of caution to offer:

Since syphilis is a disease which tends to relapse after a longer or shorter period of freedom from symptoms, he said, a prolonged observation of a large group will be needed to confirm the promise which is held out by the first group of patients.

Since that time more than 500,000 syphilitic patients have been treated with penicillin. The Executive Committee of The American Venereal Disease Association recently issued (1950) a brief summary concerning the results of more than 300,000 cases of syphilis submitted to penicillin therapy. There is complete agreement; this report states that the administration of penicillin alone results in the rapid disappearance of *Treponema pallidum* from lesions and a rapid healing of skin, bone and mucous membrane lesions. At the present time more than 4,000 patients with primary and secondary syphilis treated with varying amounts of penicillin have been observed for periods longer than two years. With 78 to 90 per cent of these patients all the reactions and tests were negative indicative of their complete cure. Thus there is a strong conviction that the great majority of the

patients with early syphilis can be cured by penicillin therapy. There is also conclusive evidence that penicillin alone, either before or during pregnancy, gives results approaching perfection in the prevention of infantile congenital syphilis. The striking fact is that syphilitic patients (with primary and secondary infection) treated with penicillin are rendered non-infectious in a matter of hours, and that the majority remain so.

Not less hopeful are the results of the treatment of chronic neurosyphilis. Often syphilitics might become blind, owing to atrophy of the eye nerve. Dr. Stokes and associates reported a striking improvement in the case of a patient who was losing his eyesight due to this disease. He was treated with large doses of penicillin (2,400,000 units) and showed remarkable improvement. It is already clear that penicillin is our most effective medication in both early and late neurosyphilis. Even in the treatment of paresis the evidence seems more and more convincing that penicillin is effective in many cases of this condition. Many patients with late latent syphilis are being treated with penicillin in this country because of the safety of the drug and the belief that an agent which is effective on early and late active syphilis will also be effective in the latent form of this disease.

It is in the field of syphilis that penicillin therapy has been the most dramatic. For here is a wide-spread infection, dreadful because of its complications, resistant to many other treatments and which responded promptly to penicillin.

RAT-BITE FEVER

Rat-bite fever is a peculiar, and relatively rare, disease of man. It develops, as a rule, after the bite of a wild rat, but a dog, cat, white rat, or even a weasel may be the cause. This disease is not new. Indeed, it has been known for more than a thousand years in India and possibly was brought into

Europe, as well as into this country, from that land. There are two types of rat bite fever. The Oriental form is caused by the germ called *Spirochaeta morsus muris*. The infection prevalent in the United States is induced by a microorganism *Streptothrix muris rattis*. No effective treatment was known for this disease; sulfa drugs proved to be of no help.

Drs. W. A. Altmeier, H. Snyder and G. Howe of the University of Cincinnati Medical School treated three cases of small children who were the victims of rat bites with penicillin.

In one case a white child, ten months old, had been bitten on the left hand and right foot by a large brown rat two weeks before she was admitted to the hospital. Within ten days she developed high fever and a skin rash, which began on the face and spread rapidly over the whole body. At the time of admission she was acutely ill with a temperature of 103.6°. A red rash was distributed generally over the body, including the palms of the hands and soles of the feet. Amazingly enough, the tooth marks from the rat bite did not appear inflamed. Her lymph nodes were somewhat enlarged. The blood culture was reported positive for *Streptothrix muris rattis*. At first the child was given sulfadiazine, but without any beneficial effect. The sulfa treatment was then discontinued and penicillin therapy was started. After she was given 300,000 units, her temperature went down to normal and she promptly recovered.

An even more striking case was reported by Dr. Edward S. Petersen and associates. A real estate appraiser was bitten by a rat and gradually developed endocarditis caused by *Streptobacillus moniliformis*, a fatal disease for which no cure was known. Penicillin therapy brought down his temperature and eliminated the infection.¹⁶

There no longer seems to be any doubt that penicillin is effective in the treatment of rat bite fever.

DIPHTHERIA AND VINCENT'S ANGINA

The great danger of diphtheria lies in the peculiar property of this germ to produce poisonous substances. The toxins of *Corynebacterium diphtheriae* affect the heart. Small children are particularly vulnerable to heart failure from this disease. Thus the present treatment of diphtheria is directed toward neutralization of the toxins. The antitoxin serum is an important part of the modern therapy. Although the germ of diphtheria is very susceptible to penicillin, the drug is not capable of neutralizing the poisonous substances which the germs produce. Therefore penicillin is given to the patient as a supplementary treatment (in conjunction with the antitoxins) to destroy the virile germs. In some cases a blood infection may take place. In such instances penicillin renders an important service by abating the secondary infections of streptococci or staphylococci.

The condition known as Vincent's angina consists of an inflammation of the mouth, tonsils, and throat. It usually begins as an infection of the tonsils (tonsillitis) or with a sore throat, and soon leads to the formation of yellow membrane resembling that of diphtheria. This infection is caused by a microorganism called *Borrelia vincentii*, which is highly susceptible to penicillin. The results of penicillin therapy have been excellent. Denny and his associates have stated that penicillin is the most effective drug yet known in the treatment of this disease. The patients were given intramuscular injections every two or three hours (from 10,000 to 20,000 units) for three or four days and completely recovered from the infection.

Even the local application of solutions containing penicillin may cure the disease. Dr. A. E. Strock had his patients hold penicillin solution in the mouth for forty to sixty minutes.¹⁷ Dr. G. F. Joseph reported that according to his

observation, it suffices to give the patient only two intramuscular injections of penicillin (50,000 units each, three hours apart) Highly effective are the penicillin trochées as they are chewed by the patient, penicillin is released and gradually destroys the germs

Although Vincent's angina is not, as a rule, a dangerous disease, in many instances it leads to complications of the tonsils or the ears. With penicillin this disease is no longer a problem for the medical profession

INFECTION OF SINUS AND TONSILS

To the numerous sufferers from infections of sinus and tonsils penicillin may bring relief, if not a complete cure In many instances penicillin has proven to be of high efficiency, perhaps more so in cases where the infection is acute, accompanied by the fever and discomfort common to these diseases

While the drug may be less effective in chronic sinus and tonsil infections as a part of regular treatment it still affords great relief to sufferers In acute cases of sinus or tonsil infections the patient is simultaneously given intramuscular injections and local applications of penicillin In case of sinus infection the drug may be given in the form of a suspension, a few drops of which are introduced into the nasal cavity When the tonsils are infected trochées or tablets of penicillin dissolved in the mouth must supplement intramuscular injections of the drug

OTHER DISEASES

In many other infectious conditions which have not been reviewed the effectiveness of penicillin therapy is determined by the type of the germ which causes the disease In trachoma, which is caused by a virus like organism, in acute rheumatic fever and in rheumatoid arthritis as well as in ulcerative

colius penicillin offers little therapeutic effect. In many cases of osteomyelitis, particularly those caused by staphylococcus, the penicillin therapy is quite effective. In erysipelas, penicillin gives excellent results. Topical applications of penicillin in cases of acne vulgaris and impetigo contagiosa have proven very effective. Patients with relapsing fever may or may not benefit from penicillin therapy. The drug has been ineffective in controlling the progress of chronic nephritis, but it may be helpful in the treatment of the acute form of this disease. Ludwig's angina may be successfully treated with penicillin alone or in combination with sulfadiazine.

Finally, penicillin has found its place in the treatment of acute conjunctivitis (2,500 units per cubic centimeter every five minutes). In the opinion of Dr. A. Sorsby, penicillin is so effective against all the common germs of acute infection of the mucous membrane of the eye that the drug may, in his opinion, completely replace the sulfa compounds in the treatment of these conditions.

When Dr. Fleming discovered his now famous mold and extracted the first crude penicillin, he never visualized the potentialities of this substance as an antimicrobial agent on such a huge scale. Even when the first clinical observations indicated that this drug had unique antibacterial properties, neither Dr. Florey and his associates nor their American colleagues ever imagined that penicillin would find application against so great a host of diseases. For, although the drug is not a cure for all infections, and has considerable limitations in its power to fight and to destroy pathogenic germs, its therapeutic usefulness and effectiveness is truly remarkable.

It is hardly possible to approximate how many lives penicillin has saved. The number may run into hundreds of thousands. Certainly it will save many more millions in the future. Strange, indeed, that so much good should come from

an accidental discovery of a mold. If the mold had been lost in the routine cleaning of Dr Fleming's laboratory, would it ever have been discovered by some other scientist? Possibly so but it might have taken many years during which time many lives would have been lost.

There is no such thing as an accident in scientific discovery Pasteur once observed. One may agree with him, and yet there seems to have been something Providential in the fact that this mold was sent to St. Mary's Hospital laboratory and was investigated by Alexander Fleming.

9

The Story of Patulin—Clavacin

ONE FALL, in the famous laboratories of the Imperial Cancer Research Fund in London, there was considerable excitement. Usually peaceful, occupied with routine investigations of the problems of heredity in relation to cancer and tissue diagnosis, this institution was suddenly thrown into a turmoil.

Was a new discovery on cancer about to be made? Had a new cure developed at last? Unfortunately not. Yet there was good reason for the confusion and excitement that had upset this conservative British laboratory. An accidental discovery had been made, one in no way connected with the fundamental tasks studied here.

It started with an ordinary occurrence. Dr. W. E. Gye, director of the laboratory, had a bad cold, a sort of grippe, an influenza-like cold caused by a virus. At this time Dr. Gye was conducting some experiments with an extract of a mold. His work deserves an explanation:

There is a theory, which is not without its supporters, that an infinitesimal virus-like organism is the cause of cancer growth. Since this type of ultramicroorganism cannot be seen through the ordinary microscope, its presence must be established by special means. The theory is not accepted by the

majority of scientists for they consider that there is no foundation for the hypothesis that cancer is of infectious origin. They argue that no one has been able to prove unequivocally that the disease is caused by a living agent. The fact that cancer can be produced by various chemical agents seems to leave little room for the infection theory. However a number of medical men of high rank stubbornly hold to this theory among them are Dr Peyton Rous of the Rockefeller Institute for Medical Research and Dr Gye of London.

A few years ago Dr Gye made the sensational announcement that he had isolated an agent that initiated some malignant growth. He insisted that the organism always present in cancerous tissue causes a growth when injected into healthy animals. Gye's microorganism was thoroughly investigated by other scientists. The latter proved that, while it might be present in cancerous tissue its presence was purely accidental. It is not itself the cancer agent if such an agent does exist. However despite the negative conclusions reached by others Dr Gye continued to adhere uncompromisingly to his own theory.

When Dr Gye learned of the discovery of penicillin it was only natural that he should attempt to apply the drug to cancer. If penicillin could destroy many types of germs it might be able to liquidate the hypothetical agent of cancer. He began to give penicillin to cancerous animals. He treated many mice and rats but to no avail. Tumors grew entirely unaffected by penicillin. There was little doubt that the drug was powerless to stop tumor growth even for a short period.

But Dr Gye did not accept defeat. To him the negative results with penicillin therapy were unconvincing. He realized that penicillin destroys only certain types of germs; it does not have the power to affect the virus like of cancer. But other molds might exist which have the magic power to kill cancerous cells. He continued to in

vestigate, in the hope that he would find one to meet his requirements

One day Dr Harold Raistrick professor of biochemistry in the London School of Hygiene and Tropical Medicine, informed him of the discovery of a mold belonging to the species *Penicillium*, a distant relative of the now famous *Penicillium notatum*, which produced a substance quite different from penicillin itself. The mold had been obtained by Dr Raistrick some years before from Baarn, Holland, and had been identified as *Penicillium patulum* (Rainier). The substance produced by this mold was accordingly called *patulin*. Patulin is a colorless beautifully crystalline substance soluble in water and most organic solvents. When examined in the test tube, it showed an inhibiting effect on both Gram negative and Gram positive bacteria.

However, animal experiments with patulin had offered little hope that the drug could be introduced into the blood stream, for it had a harmful effect on the organism. It was toxic for white blood cells, when injected into a mouse it killed the animal, damaging the kidneys and the liver. Patulin appeared to be of no use in medical practice. Nevertheless, it was sent to Dr Gye for trial on cancer.

So Dr Gye tried patulin on cancerous animals. He found that the drug was powerless to arrest malignant growths. At this distressing point in his research, the English scientist caught cold. Perhaps a disgusted devil-may-care mood was induced by the cold and prompted him to use the drug on himself, perhaps it was the adventurous spirit of a true experimenter. Whatever motivated his decision Dr Gye tried the drug in the form of a nasal douche. Next morning I was completely well and back at work, he reported.

Patulin was then tested on members of Dr Gye's staff. Dr Gye reported at length on some of the cases he treated. Among them was the striking case of Dr D. P. In November of 1942

he was kept indoors for five days with a violent infectious cold. His nasal passages were blocked and he sneezed continuously. On November 24 his nose was running and he felt neuralgic pains in the bones of his face. On the same day he gargled and douched his nasal passages with patulin four times during the day. The next day he recorded: Woke with nasal passages clear and dry. No cough. Slight huskiness of voice. No further treatment. Returned to work. Commenting on the case of his assistant who suffered frequently and severely from colds Dr Gye said: Many people regard the common cold as an annoying inevitable minor illness suitably treated with contempt. Those few who are subject to frequent attacks each of which lasts several weeks and makes life a misery do not take such a lighthearted view.

The success of these treatments convinced Dr Gye that patulin was of therapeutic value for the common cold. Inspired by his report an extensive investigation was initiated by Dr W A Hopkins surgeon commander H M Royal Navy on one hundred eighty patients at a Naval Depot. Ninety five patients suffering from colds were treated with patulin. Fifty five of them recovered the next day. The other eighty five served as control and were not treated. Only eight of these recovered by the following day.¹

The results of these experimentations with patulin created a sensation in England where the common cold is so prevalent it is regarded almost as an inseparable part of English life. Hope blossomed that at last medical science had found a remedy for this ailment. However the clinical investigation by Dr C H Stuart Harris and Dr A F Francis threw a shadow of doubt over the effectiveness of patulin as a cold cure.²

It is now generally conceded that the common cold is not a disease caused by a single agent. There are it is believed two or more virus like ultramicroorganisms as well as some

of the common bacteria which are responsible for the invasion of the nasal and pharyngeal mucous. Perhaps patulin is active only against certain of the germs which cause the common cold and is powerless against others. Since there was considerable demand for patulin an extensive investigation was conducted by the Clinical Trials Committee of England. Six hundred sixty eight patients suffering from acute forms of colds were treated with the drug. The solution used was rather weak only 1 in 10 000. 680 patients served as control. Very little difference with regard to the results was observed between the treated and untreated patients. In some instances there was an improvement and even a dramatic cure but on the average the amelioration of the symptoms was so insignificant that the conclusion was unfavorable as to the therapeutic value of patulin.² The British Medical Research Council concluded that "No evidence was found that patulin is effective in the treatment of the common cold."

The story of patulin did not end there. Dr. Florey and Dr. Chain came across another mold also a member of the *Penicillium* group. They discovered that this mold which they named *Penicillium claviforme*, produced a germ-destroying substance. They promptly isolated this drug in crystalline form and named it *claviformin*. But when they began to delve deeper into the chemistry of this new antibiotic they found that the chemical structure of claviformin as well as the chemical formula was exactly that of patulin. Moreover their investigation in the test tube convinced them that they were dealing with the same antibiotic—patulin.

While the British scientists were confronted with the similarity of two antibiotic substances produced by different molds and were trying to find the proper identification of the newly discovered drugs American investigators came across a similar problem.

If you leave a cup of very sweet tea in a dark corner of

the room for two or three days you will find on the surface a mold usually white at first and then blackish. Under a magnifying glass the mold will appear as a characteristic fruit tree like small plant. From one rounded end stalks radiate. The mold differs from *Penicillium* not only in structure but also in habits. It prefers a slightly higher temperature flourishing at body temperature about 98.99° F. It grows best on material rich in sugar. It does not dislike acid as does *Penicillium*, rather preferring in fact a medium on the acid side. If you wish to catch this mold in your teacup be sure to sour your tea with lemon. Dusty air is full of spores of this mold the *aspergillus*.

One member of this family of molds *Aspergillus clavatus* produces a substance which possesses antibacterial properties. It was this substance named clavacin that Dr. Waksman discovered at almost the same time that Florey and Chain announced the isolation of claviformin. The drug was soluble in water and alcohol and was very stable if kept in a slightly acid solution. It was remarkably effective against many Gram positive as well as Gram negative germs. It was not only capable of inhibiting the multiplication of the bacteria but also killed them. This meant that the drug was not only bacteriostatic but bactericidal as well. Moreover it seemed capable of destroying some fungi which cause skin infection.⁴ Yet when the drug was tested *in vivo* on animals it proved quite toxic. It destroyed white blood cells and when injected into the blood stream caused damage to the liver and kidneys of the animals. But the most intriguing finding was the fact of the similarity of this drug to patulin and claviformin. By chemical analysis and X ray crystallography Dr. Chain demonstrated that *all three substances were identical*.⁵

This new disclosure revealed what had already been suspected—that an identical chemical substance may be produced by two or more different molds. These might belong

to different species and yet be capable of producing an antibiotic substance of the same chemical nature and of similar biological properties. Hence these three drugs, each bearing different names, patulin, clavacin, and claviformin, are actually the same chemical agent.

10

Streptomycin, Neomycin, and Fradycin

It took Dr Fleming ten years to bring his discovery of penicillin to a successful conclusion that is to a point where it was at last recognized by the medical profession. Ten years of struggle and of endless appeals to his colleagues for assistance in proving that a common mold was capable of producing a substance which destroys some of the most hostile and dangerous microbes.

The story of streptomycin—a substance which is effective in many instances where penicillin is powerless—is even more striking in its development. For the microorganism which produces streptomycin was captured by Dr S. A. Waksman thirty years ago in 1919 when Waksman isolated a microorganism known as *Streptomyces griseus* or *Actinomyces griseus* from the soil.

Waksman is one of the most ardent devotees of the new science of antibiotics which is already playing so important a role in the fight against infectious disease. Enthusiastically he visualizes new discoveries which will bring not a partial but a complete solution of the age-old problems of medical science by utilizing the amazing ability of molds and other microorganisms to create chemical weapons against germs.

For a number of years he has been working in his laboratory at Rutgers University trying to capture some of the most prolific of these small organisms. He has discovered several antibacterial substances which may prove of considerable value to medical practice. But the amazing fact is that while he and his associates have spent years testing numerous microorganisms for their antibiotic properties only quite recently was the discovery of streptomycin made. Actually it was discovered by Waksman and his associates in 1944 *twenty five years after they had originally captured it*.

The soil and the air are full of peculiar microorganisms that are neither bacteria nor molds. They resemble both. They may be called with equal propriety bacteria like molds or mold like bacteria. Some prefer to call them super bacteria fungi. The name of their genus is *Actinomyces*. To this group of bacteria molds belongs *Actinomyces griseus* which produces streptomycin. These bacteria molds grow in the form of branched filaments or threads forming a true mycelium like that of a common mold. When this organism is well developed the surface of the colony has the powdery look which we associate with typical molds. What then differentiates them from common molds? How can one distinguish an actinomyces from other molds? Their body the mycelium is always made up of fine very narrow filaments which as often as not are fragmented. The word actinomyces means in fact ray fungi. They may be white gray or any other color. They are a large family and reveal considerable variety in appearance. Unlike common molds they are sensitive to acid reaction and will not grow in an acid medium. If your tea was soured with lemon there is very little chance that this mold will grow in your cup. But a bowl of chicken broth most surely will catch some of these fast-growing super bacteria.

The mold *Streptomyces griseus* produces the drug more effectively when it is cultivated in an agitated and submerged

medium than in a surface and stationary tank. Figuratively speaking the mold likes movement. It requires also a special nutritive substance to make it grow rapidly. Meat extract provides this stimulating factor. But corn steep liquor produces a similar, though less enhancing effect upon the mold.

Streptomycin has been prepared in crystalline form but as yet its chemical formula is not known. (It occurs as an organic nitrogenous base.) It is soluble in water and in acid solutions. It is more resistant to heat than penicillin. In powder form it may remain intact at room temperature for as long as eight or ten months. Yet it is better to keep it at a temperature below 20° C. The preparations which are on the market contain a million units in one gram. The antibacterial activity of the drug is measured not chemically but by the inhibiting effect on the growth of some germ (*Esch. coli*).

When streptomycin is given to a man by injection it is readily absorbed by the system. Let us assume that a dose of 500 000 units (0.5 gram) is administered by intravenous injection. Very soon as much as 30 units per cubic centimeter may be found in the blood. And although the drug is excreted by the kidney quite rapidly its disappearance from the blood stream is much slower than that of penicillin. Even six hours after the injection the blood may still contain as much as 10 units per cubic centimeter of blood. The most satisfactory method of administration of streptomycin is by way of intramuscular injection. A hundred thousand units given every three or four hours will keep the level of the drug in the blood at about 3-4 units.

It appears that streptomycin cannot be given by mouth. When taken in the form of tablet it is not absorbed at all and is eliminated intact in the feces according to Anderson and Jewell.

Unlike penicillin streptomycin is of considerable toxicity. It

does not affect the red and white cells and does not harm the bloodforming system. Unfortunately, it does produce an irritation of the kidney which may result in the appearance of blood and albumin in the urine¹. The most grave complications are due to the toxic effect of the drug on the central nervous system (the eighth cranial nerve). Vertigo is the commonest reaction in patients treated with streptomycin; this was observed in nine out of ten persons. The vertigo may persist for days or weeks after the treatment is discontinued. In some patients who have received streptomycin for a period longer than three weeks deafness may develop. As a rule it is of a temporary nature, but in rare cases the hearing may be impaired to some degree for the rest of their lives. It is what we call *neurotoxic* reactions that are the most disquieting feature of this therapy.² However, when the treatment is not very extended and is limited to a three-four weeks period and the dose given is relatively small (1 gram per day) the toxic reactions may be of a relatively harmless and temporary nature.

The hazards of streptomycin toxicity have been somewhat reduced by the chemical modification of streptomycin to a new form called *dihydrostreptomycin*. This modified antibiotic retains all the antibacterial properties of the original drug but is much less toxic although even this new form of streptomycin is not devoid of all toxic reactions and should be applied with some care.

The characteristics of streptomycin are strikingly similar to those of penicillin. However, they differ in some respects. Streptomycin is much more effective against Gram negative and acid fast bacilli. Hence some of the bacilli against which penicillin is ineffective are attacked successfully by streptomycin. Among these penicillin resistant bacilli is the tubercle bacillus. It is in the treatment of tuberculosis that the usefulness of this substance seems to be particularly promising.

STREPTOMYCIN IN TUBERCULOSIS

A normal seventeen months old child of good constitution became gravely ill. For a month a high temperature of 101-103° weakened him. The child began to lose weight and refused to eat. His parents both healthy showed no signs of tuberculosis. In fact, no one on either side of the family was afflicted with this disease. The roentgenogram of the child's chest was not entirely conclusive although some infection was apparent. A more thorough examination revealed numerous tubercle bacilli (*Mycobacterium tuberculosis*) in the stomach. Immediately a streptomycin therapy was initiated and the child was given about 1 000 000 units a day for thirty days. After twenty days of treatment he manifested all the symptoms of recovery. His temperature went down to normal. He gained weight and his appetite returned. The X ray examination indicated that the lungs were completely cleared. The case was reported recently by Drs. Heworth N. Sanford and Donald E. O'Brien of the Presbyterian Hospital, University of Illinois.

This child was not the only one so treated. Three other children among them an infant of seven months recovered from an unquestionable pulmonary tuberculosis after *one month* of streptomycin therapy.

These are dramatic cases. Not only do they suggest an effective remedy for this disease may be available but also that the drug itself is not harmful. If an infant was able to tolerate as large a dose as a million units per day for thirty consecutive days without ill effects and regained health there is hope that streptomycin will be of use in the fight against a stubborn affliction that is as yet far from conquered.

However, in spite of this encouraging report the therapeutic value of the drug in tuberculosis is still under discussion.

by medical men. The experimental and clinical evidence, although very encouraging, is so far by no means conclusive. For not only is the problem of tuberculosis much more complicated than that of other infectious diseases, but the tubercle bacillus has peculiar properties which we seldom meet in the bacterial kingdom.*

Naturally, the question arises whether or not streptomycin can penetrate the protective capsule of the tubercle bacilli to destroy them. Experiments in the test tube have given no evidence that the tubercle bacilli are destroyed by streptomycin. That is the conclusion at which the investigators have arrived. But streptomycin does inhibit their growth, and impedes their proliferation.³

The general assumption is that streptomycin behaves in the test tube as a bacteriostatic agent that retards the development of the tubercle bacilli. In the human body it acts in the same way. As Dr. Emile Bogen stated: 'Streptomycin is a retardant, rather than a bactericidal agent. The tubercle bacillus can be kept from growing without actually being killed, over a wide range of concentrations of streptomycin. But this retarding action may be of considerable importance in saving lives.'⁴ The experiments on animals corroborate his statement.

The first streptomycin experiments on animals were instituted at the Mayo Clinic in 1946. Drs. H. Corwin Hinshaw and William H. Feldman used thirty-two guinea pigs to test the new drug. Fourteen were inoculated with the culture of tubercle bacilli, then injected under the skin with streptomycin. The dose varied from 1,800 to 6,000 units per day, divided into four doses. The treatment continued for more than fifty days. The remaining 'control' animals were infected with tubercle bacilli, but no treatment was given. The

* See pages 61-62 for the earlier description of tubercle bacillus.

untreated animals all developed pronounced tubercle lesions, while the animals submitted to streptomycin therapy were free, or almost free, from tuberculosis infection

Simultaneously, similar experiments were conducted at the National Institutes of Public Health, Bethesda Maryland Drs M I M Smith and W T McCloskey treated with streptomycin twenty five guinea pigs infected with tubercle bacilli The animals were given 5 000 units per day for about sixty days The results were identical to those obtained at the Mayo Clinic The appearance of tubercle lesions were considerably retarded or prevented altogether⁵ A number of other investigators have recently repeated similar tests either with guinea pigs or mice with encouraging results

The most convincing investigation, however was reported recently from the Pasteur Institute in Paris C Levaditi and A Waisman infected mice with one milligram of tubercle bacilli quite a large amount They conducted two series of experiments In the first series twenty four mice received 1,000 units of streptomycin while seventeen mice were used as controls In this series the treatment was started simultaneously with the inoculation the animals received their first dose of the drug on the same day that they were infected with the tubercle bacilli In the second series eighty mice were subjected to the streptomycin therapy with thirty seven mice serving as controls Half of the treated animals were given streptomycin only twelve days after they had been infected

The results obtained by the scientists at the Pasteur Institute convinced them that *streptomycin is one of the most effective agents for the treatment of experimental tuberculosis in mice* Levaditi and Waisman made a detailed examination of the behavior of the tubercle bacilli in the bodies of the mice submitted to streptomycin therapy The drug does not destroy the germs directly but checks their multi

plication. The bacilli lose some of their aggressive vitality and fall easy prey to the white blood cells, the phagocytes. Thus streptomycin is a valuable aid to the defense mechanism in the battle between the organism and the invaders. By restraining the activity of the tubercle bacilli, the drug permits the organism to fight the germs successfully. Consequently, the tissue where the bacilli were injected presents the typical picture of a healing process, a cicatrization which is able to withstand invasion by the bacilli. But there is not a complete cure, no radical sterilization of the germs. When the treatment is discontinued the infection may develop again and the animal may die of tuberculosis.⁶

From these investigations of experimental tuberculosis it is apparent that *streptomycin is not a cure against this disease. Alone it cannot fight the infection successfully and cure completely, but it renders valuable service to the defense forces of the organism by depleting the vitality of the germs.*⁷

As soon as the results of experimentation on animals proved encouraging, an extensive clinical investigation was initiated at the Mayo Clinic. The first report on one hundred patients treated with streptomycin was published in 1946 by Hinshaw, Feldman, and Pfuete, and embraces two years of clinical work.⁸

Various types of tuberculosis were treated. The most hopeless cases of miliary tuberculosis, where the infection is generalized, received intensive streptomycin therapy. Out of twelve patients affected with generalized tuberculosis and subjected to treatment, six died. The rest were alive at the time the report was published. One patient showed pronounced improvement after two months of treatment. This is an enormous achievement, considering the gravity of the illness.

Four patients who developed meningitis, the most dangerous complication of tuberculosis, showed signs of improve-

ment In fact, the response to streptomycin on the part of these patients was unmistakably good, consistent and prompt The improvement began after one or two weeks of therapy The temperature went down to normal In all four cases the return of a sense of well being and the disappearance of headache was manifested These patients were treated for six months Three of them remained in good condition for two or three months after the injections were stopped In one case the infection returned with new force after a month consequently he was given new and prolonged treatments with streptomycin

Physicians at the Mayo Clinic also treated a number of cases of lung tuberculosis In about 30 per cent they noted an unquestionable improvement which manifested itself in the closing of the lung cavities and the disappearance of tubercle bacilli from the sputum of the patients

It is wise to recognize as clearly as possible the limitations which are imposed on the treatment of tuberculosis by antibiotic agents Hinshaw and his associates concluded in their report and especially to emphasize the limitations and shortcomings of streptomycin Unfortunately tuberculosis in human beings pursues an insidious course often it does not produce symptoms until irreversible destructive changes have been wrought in the tissues Actual healing in tuberculosis must be accomplished by the slow process of resorption fibrosis and calcification and the role of an antibacterial drug is that of blocking the paths for extension of the disease while these healing forces are operating *

In human beings tuberculosis is much more complicated and resistant to treatment with an antibiotic agent than in animals The infection is often of long duration for the germ is well adapted for the battle against the defense forces of the organism The value of streptomycin is its ability to help the

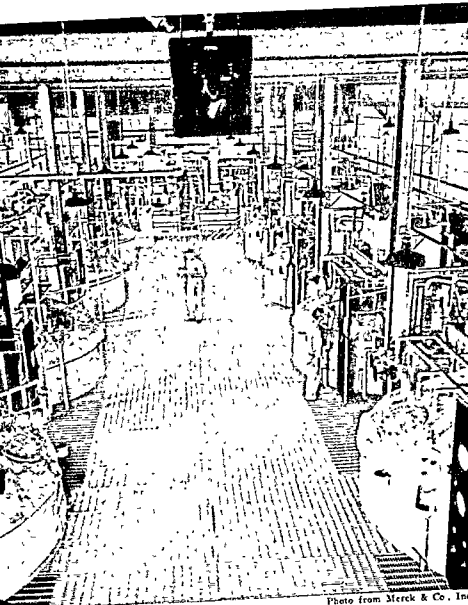
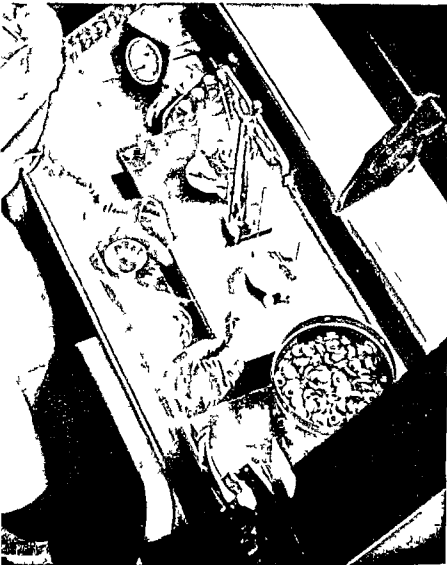


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In their recent review of streptomycin therapy in tuberculosis Drs. Karl H. Pfuetze and Marjorie M. Pyle stressed the fact that this drug "is accepted as the most effective treatment known for ulcerative lesions of the larynx and tracheobronchial tree" ¹⁰ In some cases they were able to arrest tuberculosis of the kidney for more than two years. However, they do not claim a permanent cure of this deadly disease. And the drug proved of some help in tuberculosis of bones and joints. "In the treatment of pulmonary tuberculosis," they stated, "the necessity for careful selection of patients must be emphasized. We believe," they continued, "that streptomycin should not be used in minimal pulmonary tuberculosis, or for any other tuberculosis condition for which other satisfactory treatment is available."

The drug is of considerable usefulness when a tuberculous patient is operated upon. Drs. Edward J. Beattie and Brian Blades pointed out that "streptomycin is a valuable adjunct in the surgical treatment of pulmonary tuberculosis. The immediate complications are decidedly reduced." Even when the case was very grave and the surgical operation was a desperate risk, still, due to the help of streptomycin therapy, there were no deaths ¹¹

While in chronic tuberculosis of the lungs the results are not always satisfactory, streptomycin therapy often produces a dramatic effect in tuberculosis of the larynx and ear.

"The response to streptomycin in tuberculous laryngitis was almost miraculous," said Dr. Rufus F. Payne, Superintendent of the Battey State Hospital in Rome, Georgia, in his report on six hundred fifty tuberculous patients treated with the drug.

"Patients have been enabled to talk in normal tones, even

though they had not spoken except in a whisper for three years . We have other patients who have not been able to swallow small amounts of water Within three weeks after they started taking streptomycin, they were eating a normal diet, completely free of pain "

Streptomycin seems to be most effective in recent and acute infections When the germ is very active and aggressive but the organism offers stubborn resistance to the bacterial invasion, the drug can render invaluable service to the patient This fact was emphasized in the recent report of the Clinical Subcommittee of the Committee on Medical Research and Therapy of the National Tuberculosis Association

' Streptomycin appears to be most effective,' the report stated, ' in the treatment of recent, acute, fairly extensive and progressive pulmonary tuberculous lesions Its use is particularly recommended in the treatment of tuberculous pneumonia The drug should not be used for a period longer than two to four months A longer application of the drug may reduce its usefulness For this reason it should be administered in emergency cases when the infection is progressing rapidly and the danger is acute ' It is imperative that streptomycin be used for a period during the course of the disease when the greatest benefit can be expected The Committee looks with disfavor on the practice of utilizing streptomycin prior to institutional care ' By this they mean that other therapeutic measures should be taken prior to the administration of the drug

In types of tuberculosis where heretofore medical science could offer neither help nor hope, the merit of this drug is particularly significant In cases of miliary tuberculosis when the germ is spread by the blood stream into every organ of the body, when the infection is generalized and often attacks the most vital organs, such as the brain, where the infection has been 100 per cent fatal streptomycin offers some hope.

even a chance for recovery. Although the drug itself does not effect a cure in these grave forms of tuberculosis it gives the defense forces of the organism an opportunity to fight for life again. The figures so far available indicate that about half the cases affected with military tuberculosis have received a new lease on life. They are not cured, but do have a chance to survive, and gradually to conquer this terrible disease.

"There is no doubt that streptomycin has saved and prolonged hundreds of lives," concluded Dr. Payne.

On the other hand, "Streptomycin is not to be regarded as a substitute for other and proved effective forms of treatment of tuberculosis" (Hinshaw)¹² It should be used as a supplementary, often powerful, therapy in conjunction with all present methods of treatment.

Unfortunately, the tubercle bacilli rapidly acquire resistance to this drug. And that is one of the greatest handicaps in streptomycin treatment of tuberculosis. Dr. M. Pyle of the Mayo Clinic found that tubercle bacilli isolated from patients before they were submitted to this treatment differed considerably in their tolerance to the destructive action of the drug. Drs. Youmans and Williston of Northwestern University corroborated this finding. Of fifty-seven cultures of tubercle bacilli isolated from patients they found that nearly half (43.5 per cent) were considerably resistant to the action of streptomycin. Some of these germs were so resistant that even as high a concentration as 100 units per cubic centimeter was not capable of inhibiting their growth. The peculiar ability of the bacilli to become resistant to streptomycin may account for the number of failures in the treatment of this infection.

STREPTOMYCIN IN UNDULANT FEVER

A farmer living in southern Maryland complained of fatigue, sweating, stomach-ache, and severe headache. A well-developed man in his early forties, he displayed no apparent

symptoms of any illness. In fact, he was inclined to disregard his condition, stating simply that he was "a little damp," and mentioning briefly that he had 'neuralgic headaches and stomach upsets. Only under the physician's queries did he admit that his severe fatigue had persisted for many months, that he had had a stiff elbow for a month or two with no swelling or redness, and that he had suffered repeated headaches in the mornings. His sexual desire had lessened. He had a slight fever, his temperature ranged from 99 to 100°. These conditions had existed for about a year before he consulted the physician. His blood culture yielded a germ known as *Brucella suis*. A diagnosis was made of undulant fever, otherwise brucellosis. He was treated with streptomycin combined with sulfa drugs and according to the report by Drs. H. C. Harris and P. C. Jett, promptly recovered.¹³

In this country alone about 60,000 people succumb to brucellosis every year. Until now, no specific and effective treatment was known. Although the infection as a rule is not dangerous, it is persistent and in acute cases may be fatal. Edsel Ford was killed by undulant fever.

How does one contract this infection? In most cases the person does not even know the origin of his illness. The germs are present in the milk of cows infected with brucellosis. If the milk has not been pasteurized the disease may be transmitted. Even from touching a goat, a horse, a cow, or a pig which has this disease, there may be a possibility of getting brucellosis.

Also known as Bang's disease, brucellosis causes infectious abortion in the cow, goat, or sow. It is characterized by an inflammatory condition of the female reproductive organs, which results in the expulsion of the immature young. Sir David Bruce, British medical officer, first discovered the germ.

There are various types of brucella. Most common in this country is *Brucella abortus*, which attacks calves, also *Brucella melitensis* which is found in goats and, when transmitted to man, causes Malta fever. The third type of brucella was recovered from swine by Traum, and is called *Brucella suis*. The Maryland farmer was infected with this last type of brucella which he had probably caught from his pigs.

When streptomycin was discovered, medical science wondered whether it might be used for the treatment of undulant fever. But, as usual in such an investigation, animal experiments had to precede application of the drug to human beings. That is the law of modern medical science. To test the germ of brucellosis on mice or guinea pigs would be a long and costly procedure. A simpler technique had to be found. Waksman and his associates at Rutgers University developed an amazingly ingenious method for testing the efficacy of streptomycin against the undulant fever germ.

Using fertilized eggs containing seven day chick embryos they injected a fresh culture of brucella (*Brucella abortus*) taken from a human patient suffering from undulant fever into the embryo (via the yolk sac).¹⁴ Uniformly, all the chick embryos became infected. The microorganism destroyed them with the accuracy of a stop watch. Half the embryos were dead within six days; in nine days all had perished. Thus the scientists established a test of high accuracy. If a drug could prevent the death of the chick embryos infected with brucella, it would have the power to arrest or destroy the germ.

Accordingly, shortly after new chick embryos were infected, they injected streptomycin. The embryos survived. However, when they were examined bacteriologically, the germ was found not dead; the embryo liver was full of living and active brucellas. Streptomycin had arrested the deadly march of the germ but was unable to destroy it altogether.

Thus this drug although of some efficiency against brucella was shown to be not powerful enough to treat undulant fever successfully. For this test on embryo chicks paralleled the results which would have been obtained in human patients.

In the search for a measure which would increase the effectiveness of streptomycin against undulant fever germ the scientists decided to use a combination of streptomycin and sulfadiazine. It had already been established that sulfadiazine when injected into an infected chick embryo produced a beneficial result similar to that of streptomycin.¹³ Sulfadiazine did not eradicate the infection but was capable of prolonging the life of the chick embryo. The combined streptomycin-sulfadiazine therapy gave excellent results. When a proper dosage of both drugs was used all the germs in the embryo were destroyed and the infection annihilated. Thus a new treatment of undulant fever based on precise scientific findings was offered to the medical profession.

An important center of investigation of brucellosis is the Hospital of the University of Minnesota Medical School in Minneapolis. There Dr. Wesley W. Spink and his associates of the Division of Internal Medicine have been working on undulant fever for more than ten years. They have tried every possible new method or remedy anyone has suggested to fight this infection. Recently they reported the results of their clinical investigation of the combined streptomycin-sulfadiazine therapy. They treated a number of patients suffering from an acute form of brucellosis and had excellent results. They administered streptomycin intramuscularly in doses of 0.5 gram every six hours for seven days. Simultaneously they gave the patients one gram of sulfadiazine every four hours for at least two and preferably three weeks. Summarizing the results they concluded: "The combined use of streptomycin and sulfadiazine in the therapy of patients with

either acute or chronic brucellosis has yielded more satisfactory results than has been previously obtained in the University of Minnesota Hospitals. The results are particularly encouraging, since the treatment was effective in complications of brucellosis such as subacute bacterial endocarditis.¹⁶

There is, however, one obstacle to this combined therapy. Streptomycin is relatively harmless. It does not destroy red blood cells, nor does it damage the liver or the kidneys. Yet it has been known for some time that this drug exercises some toxic effect on the eighth cranial nerve of the brain. Vertigo and other manifestations in the nervous system of the patient receiving streptomycin are explained by the peculiar effect of this drug on the eighth nerve. Some physicians (T. Hunnicutt, M. Finland and others) reported grave complications when streptomycin was injected into the cavity of the spinal cord.

Recently, Drs. Horman McCullough and C. W. Ersele encountered grave toxic reactions in several cases of brucellosis treated with streptomycin and sulfadiazine. One of these cases was truly disappointing.

A young woman while working on brucellosis in the Navy Research Laboratory in 1945 caught the infection. She became gravely ill and her ailment was diagnosed as *Brucella suis septicemia*. The germ had penetrated into the blood stream and caused blood infection. The initial illness was severe and of long duration. During the next year she was completely disabled, complaining of persistent fatigue, recurrent headaches, and multiple joint pains. She was unable to sleep and her memory lapsed. She was admitted to the hospital and simultaneously was given streptomycin and sulfadiazine. During the second day of the therapy she presented alarming symptoms of central nervous system disturbance. Semistupor, weakness of eye muscles, blurring of vision, and trembling of the lips were manifested. The strep-

tomycin therapy was halted for one week and the symptoms gradually subsided. When a new attempt was made to give streptomycin similar symptoms recurred.

The doctors trying to explain the severity of toxic reaction in this combined therapy, were inclined to conclude that the combined use of the drugs increases the toxicity as well as the clinical effectiveness of streptomycin.¹⁷

STREPTOMYCIN IN BLOOD INFECTION, MENINGITIS AND PNEUMONIA

There are some cases of blood infection where penicillin does not help. Fortunately, such cases of septicemia are rare. The bacteria belonging to the Gram positive type, such as streptococci, staphylococci and many others are subject to the destructive action of penicillin. The bacteria of the Gram negative type are as a rule resistant to penicillin. Herein lies the importance of the discovery of streptomycin. For this drug which has only a moderately destructive action on bacteria of the Gram positive type is quite effective against many of those germs which penicillin is powerless to destroy.

A young actress was admitted to the hospital. She had fever with temperatures as high as 104-105°, and complained of severe headaches. Her case was diagnosed as septicemia complicated by meningitis. Blood cultures revealed that the patient was infected by *Escherichia coli*. Her illness had begun with a mild infection of the bladder. She had paid little attention to it when one day after catching cold her temperature suddenly rose to 101° and stayed there for several days. She was treated with streptomycin given intramuscularly as well as intrathecally and gradually recovered from this serious almost fatal infection.

Meningitis caused by a Gram negative type of bacteria which heretofore offered little chance for recovery in many

instances responds to streptomycin. Keefer and his associates of Boston Hospital reported that out of one hundred cases of influenzal meningitis, 80 per cent were cured by streptomycin therapy.¹⁸ The same doctors treated sixty nine patients with blood infection caused by Gram negative bacilli, and obtained good results in more than half the cases.

In the majority of cases of pneumonia, penicillin is very effective. But there are some types of lung infection which are caused by a germ known as *Klebsiella pneumoniae*. Against this bacteria penicillin is powerless. Here again, streptomycin may help. Drs. C. A. Bishop and R. F. Rasmussen obtained good results by treating this lung infection with streptomycin. The dose should be relatively large, not less than 0.5 gram given every three hours for as many days as is necessary to eradicate the illness.¹⁹

The whole pattern of antibiotic therapy in pneumonia seems to point to a combined application of these drugs. In many acute or chronic infections more than one germ may be the causative agent. In order to achieve rapid results and to prevent spreading of the infection, many physicians are already inclined to prescribe a combination therapy of two or even more, antibiotic substances. In many instances particularly in the infections of the respiratory tract, penicillin is supplemented with sulfa compounds or streptomycin or even with both of these drugs. Often in everyday practice the general practitioner is unable to make a blood culture and establish definitely which germ has caused the infection. Yet time is a decisive factor in the success of the treatment. So long as the drugs are of low toxicity, and more or less harmless, the doctor feels that he should administer a combination of the two drugs in order to be sure that at least one will be effective against the bacteria. When a country doctor attends a patient who manifests symptoms of pneumonia, what should he do? It may be two or three days

before a blood test can be made to determine which type of germ is responsible. Since time is of the essence particularly when the patient is no longer young, he gives a combination of penicillin and streptomycin.

STREPTOMYCIN IN TULAREMIA

A young biological scientist working in the genetic laboratory of one of the universities in New York tried to mate a wild male rabbit with a female white rabbit. Quite suddenly the male animal died and the biologist was unable to determine the cause. A few days later he himself became ill. Strong chills and a fever of 103° or more were followed by a swelling of the glands. At first the doctor was puzzled by the symptoms but a blood culture revealed the *Bacterium tularense*, which causes the infection known as tularemia.

Tularemia is an acute infectious disease of rodents transmitted to man by insect bites or by contact with infected animals as in the case of the biologist. In this country, wild rabbits are the main source of infection; ticks and deer flies transmit it. Fortunately this disease is relatively rare altogether, only a few thousand cases have been reported in the United States.

Rodents cannot withstand this bacteria and the infection usually kills them quickly. Man on the other hand has considerable resistance to the infection which is usually mild yet often distressingly chronic. In some respects this disease resembles tuberculosis, with the development of numerous typical lesions which are gradually healed by the formation of fibrous substances. *Bacterium tularense* is such a small microorganism that it can penetrate unbroken human skin. Contact with the infected animal may be sufficient to transmit the infection. Although only a very small percentage of the persons infected actually die, the disease may persist for

many months, or even years, and may recur. No efficient cure was available for tularemia until it was found that streptomycin was capable of destroying the germ. This drug is a most effective remedy and, in fact, cures nearly every case.

Dr. Abraham M. Gordon, of the United States Army Medical Corps, had an interesting case of tularemia which he treated successfully with streptomycin.

A Navy man was admitted to the hospital, complaining of headache and stiff neck. His illness had started about six days before, when he came down with chills, fever, and malaise. The patient explained that a few days before the onset of the illness he had been hunting in Kentucky and had shot forty-five rabbits. He dressed six of them with his bare hands and the following day had a rabbit dinner. The patient's brother-in-law, who had helped dress the animals, was admitted to a hospital on the same day with similar symptoms. The blood cultures disclosed the germ of tularemia. Apparently one or more of the rabbits had been infected, or at least were bearers of the germ.

Immediately upon admission the patient received penicillin and sulfadiazine, but his temperature remained high, and there was no improvement. After the diagnosis was established the patient was given streptomycin in large doses, about one million units per day. The next day his temperature dropped to 96° and remained at a low level for two days. No more streptomycin was available at that time, and shortly afterward the patient's temperature rose to 105° . When a fresh supply of the drug arrived, the treatment was continued for another six days. This time there was a complete recovery.

This case is very typical indeed, for while neither penicillin nor sulfa drugs brought relief, the patient was promptly cured by streptomycin. Other cases of tularemia have also been treated successfully with streptomycin. Dr. C. S. Keefer and

his associates reported sixty three recoveries due to streptomycin therapy out of sixty seven cases of tularemia. To date this drug is the best remedy known for this infection.²⁰

STREPTOMYCIN IN PLAGUE

There was a time when plague was considered the greatest epidemic disease. It was the most devastating, the most fatal. For centuries it held the population of Europe in deadly terror. There was no cure and the only preventative measure seemed to be the isolation of the diseased persons from the rest of the population. Plague still remains the bane of the population of India. Although vaccination against it (Besredka's method) has been used extensively and consequently has reduced considerably the danger of widespread epidemics here and there plague outbreaks still occur in present-day India. Such an epidemic took place recently in the Madras province and for the first time in the history of this disease effective treatment with streptomycin was reported by the local physicians.

Less than two years ago Dr. Karl Meyer of the University of California treated mice infected with the plague bacillus with streptomycin. He reported that he was able to control the infection in about 90 per cent of the animals. The mice had suffered from the most deadly form of the disease called pneumonic plague.

When the outbreak occurred in India a large supply of streptomycin was sent there. Recently, the first report on these cases appeared in the British medical journal, *Lancet*.²¹ Several patients, mostly young people, were actually dying from this terrible disease. There seemed to be no chance to save them. Their temperatures ran as high as 106.5° accompanied by swollen glands, a typical symptom of this infection. In fact they were already in a semiconscious stupor. The cause of their illness was established by bacteriological ex-

amination. First, sulfa drugs were given, for there had been some inconclusive reports from China that they might control the infection. However, the sulfa drugs did not help the patients at the Hindupur Plague Hospital in Madras, India, so Drs. P. V. Karmachand, Anantaour medical officer, and K. Sundar Rao, chief physician of the hospital decided to try streptomycin.

The effects were striking. Within forty-eight hours after the first injections the patients regained consciousness and showed signs of improvement. Recovery was rapid and dramatic, as though the dead had been resurrected. No doubt was left in the minds of the Indian physicians, for they concluded in their report, 'Streptomycin appears to be a potent drug for the treatment of human plague.'

In his article 'Modern Therapy of Plague' (JAMA., 1950) Dr. K. F. Meyer stresses the usefulness of streptomycin in the treatment of plague. It has dramatically reduced the mortality in both bubonic and septicemic plague and is most effective in pneumonic plague. Streptomycin should be given in large doses (4 grams daily) for several days. The drug is not effective, however, in all cases of this infection.

STREPTOMYCIN IN INTESTINAL AND URINARY TRACT INFECTIONS

A girl of ten was admitted to the hospital in a very grave condition, with a ruptured appendix. She had been suffering from chronic appendicitis for many months, perhaps for years. While engaged in a game with her schoolmates she fell on her abdomen. Her fall caused the appendix to break, and in a few hours peritonitis developed. When she was examined at the hospital it was found that the infection of the peritoneum of her intestines was caused by the bacillus *Escherichia coli*, a microorganism which is often responsible for this condition. Since *Escherichia coli* is very sensitive to

streptomycin the patient was given heavy doses of the drug, not only intramuscularly, but around the area of infection as well. The results were astonishing. Her temperature went down within two days from 105° to almost normal. After ten days of intensive treatment with streptomycin a complete recovery was effected.

Not long ago peritonitis was one of the gravest complications in intestinal infections and in many instances ended in death. Infection of the peritoneum is most often caused by Gram negative bacteria which makes streptomycin such a promising agent for the treatment of this ailment. Keefer and his associates have reported that out of fifty three cases of peritonitis so treated for ten days or more thirty nine patients have recovered. Seventy five per cent of the cases were successfully treated an enormous accomplishment in comparison with the high mortality rate for untreated peritonitis.²²

Several attempts have been made to give streptomycin to typhoid fever patients. So far the results are neither conclusive nor encouraging. Keefer *et al* reported that they treated fifty one cases of typhoid fever with streptomycin and observed some improvement, but the course of the disease was not changed in spite of the very large dosage of the drug—four grams per day given intramuscularly for seven to nine consecutive days. However L. Rutstein and his associates arrived at the conclusion that if the drug was given by mouth better results might be obtained.²³

Streptomycin is beneficial when used in the treatment of grave complications such as septicemia pneumonia or peritonitis when these infections are caused by the *Escherichia Salmonella* group of bacteria.

STREPTOMYCIN IN GALL BLADDER INFECTIONS

Gall bladder infection is a common disease among Americans particularly among women. Many factors contribute

to the situation, among them wrong diet and the tensions of modern life. In the past, the treatment of cholecystitis (inflammation of the gall bladder wall) was prolonged and not always successful. Often surgery was unavoidable. However, with the discovery of penicillin and streptomycin the prospects in the treatment of gall bladder infection appear much brighter. Both of these drugs tend to accumulate in high concentration in the gall bladder, whether given by mouth or by injection. If infection of the gall bladder is caused by a germ which is vulnerable to these drugs the infection promptly disappears.

A New York doctor recently reported the case of a woman of forty nine, who was very stout and who had been suffering from inflammation of the gall bladder for a number of years. However she refused to undergo an operation although she had frequent and painful attacks. One day she became ill with an acute throat infection and ran a fever of 105° . She was given penicillin together with streptomycin in large doses. She recovered promptly from her throat infection and curiously enough from that time forward the symptoms of her gall bladder infection also disappeared.

Since gall bladder infections are often caused by Gram negative bacilli streptomycin is highly regarded by the medical profession for the treatment of this ailment. Two years ago C. S. Keefer and his associates reported six cases of cholecystitis which responded favorably to streptomycin. During the past year more data has accumulated indicating promising results with this therapy. Many a chronic sufferer from gall bladder disease may get welcome relief with this drug.

STREPTOMYCIN IN LOCAL APPLICATION

The usefulness of penicillin in ointment has been proven in a number of cases of skin infections. Only recently was

streptomycin investigated in local application Drs Leon Goldman and Milton D Feldman, from the Department of Dermatology and Syphilology, University of Cincinnati Medical School briefly summarized their results To date they said streptomycin has been observed to be of value in superficial infections with organisms which are sensitive to the drug These types of infections include of course impetigo, pustular folliculitis ecthyma and nonfungus external otitis

These investigators also pointed out that in some cases streptomycin like penicillin may produce a skin irritation like eczematoid dermatitis In one case a young man developed an acute dermatitis as a result of applications of streptomycin ointment to an ulcerative area of the skin But such cases seem to be exceptional a large number of patients were treated with streptomycin ointment with good results and without causing additional skin irritation

THE LIMITATIONS OF STREPTOMYCIN

As clinical evidence accumulated it became clear that streptomycin is of considerable toxicity In some instances the vision of patients treated with this drug manifested some impairment A condition known as scotomas (a dark spot in the visual field) developed in a number of tuberculous patients according to the report of Dr E B Thomas²⁴ There was vague blurring of vision in some treated patients This condition remained for several months after the treatment was terminated A permanent deafness might occur as the result of streptomycin therapy and did occur in a number of cases Dr A de Kleyn and Dr J B van Deirse demonstrated that this drug affects the vestibular system of the internal ear Streptomycin appears to exercise a paralyzing influence on the central vestibular nuclear area The toxic reaction of patients to streptomycin might be so great that encephalitis (an inflammation of the brain) might develop There are sev

eral cases of death from encephalitis caused by intramuscular injections of streptomycin (Campinacci). Because of its toxicity and the rapid development of bacterial resistance, streptomycin is being supplemented by other antibiotics, more effective and less toxic. Tuberculosis remains the principal disease for which streptomycin therapy is indicated (Kirby). In plague, where its toxicity is of secondary importance, it still remains the drug of choice. Urinary tract infections due to *Pseudomonas aeruginosa* and *Bacillus proteus* are possibly still best treated with streptomycin which is also used in combination with other antibiotics in enterococcic endocarditis. Although effective in undulant fever, tularemia and salmonella infections, streptomycin is nevertheless replaced by less toxic antibiotics.

In spite of its limitations streptomycin has rendered enormous help in our fight with tuberculosis and many thousands of human lives have been saved by its application in this disease.

NEOMYCIN

Dr Selman Waksman, the discoverer of streptomycin, soon became aware that this antibiotic, in spite of its potentialities, is not the solution for the successful treatment of tuberculosis. In his article (*Science*, March 25, 1949) he admitted that the problem of development of resistance remains as the major limitation of this antibiotic, especially in the treatment of tuberculosis. For some time, he and his associates have been searching for a mold which can produce a substance more effective than streptomycin as far as tubercle bacillus is concerned. It was only natural that he should direct his attention to the same group of molds to which *Streptomyces griseus* belongs. The amazing fact is that many strains and species of the genus *Streptomyces* produce substances detrimental to tubercle bacillus.

During the last ten to twelve years many hundreds of cul

tures, mostly belonging to the genus *Streptomyces*, were isolated by Waksman and his associates from soil, composts, peats and other natural substrates. All of them were tested for their activity against different bacteria. Unfortunately, only a few of the antibiotics produced by these microorganisms proved to be suitable for therapeutic application. Some were too toxic, others not sufficiently powerful. Only streptomycin answered the requirements of a nearly ideal antibiotic. But recently Drs. Waksman and Hubert A. Lechevalier announced the discovery of a new antibiotic which has, according to them, stronger activity than streptomycin against the tubercle bacilli which are resistant to this drug. They called it *Neomycin*. The organism producing this substance was isolated from the soil and was identified as *Streptomyces fradiae*. Neomycin has a broad antibiotic spectrum and is active against a variety of gram positive and gram negative bacteria. It is not active against virus and fungi. Neomycin was tested against *Mycobacterium tuberculosis*. The results were impressive. For this substance was more active than streptomycin against tubercle bacilli. As small a dose as 0.1 unit inhibited the growth of the bacterium. Moreover, the strain of tubercle bacilli known for its resistance to streptomycin (607 R) was inhibited by a slightly larger unit (0.25 unit) of neomycin. It appears to be of low toxicity to animals. However, the clinical evidence so far available, gave no indication of the superiority of neomycin over streptomycin as far as therapeutic effect was concerned.

TRADICIN

There is a peculiar paradox in the production of antibiotic substances by molds. Not unlike some bacteria, which are able to produce a substance which kills other bacteria, some molds seemingly are able to manufacture a substance which destroys other molds. We call such an antibiotic sub-

stance fungicidal or antifungal. While working with neomycin Dr E. A. Swart and associates of Rutgers University, found that 30 to 70 per cent of its activity is due to what they call 'factor λ '. Analyzing and testing this factor they arrived at the conclusion that it was actually a complex, composed of two different substances. One of these substances possessed the properties of neomycin, and was effective against bacteria. The other substance however was inactive against bacteria but very active as far as some fungi were concerned. It inhibited the growth of *Penicillium notatum*. Fradycin is a light tan powder, slightly soluble in water and quite stable at high temperature. It is believed that fradycin might be useful against some diseases in plants and cattle caused by fungi. However, no data on this subject are as yet available.

The discovery of Swart and associates is not entirely new. For before them Curt Leben and G. W. Keitt isolated from *Streptomyces* a substance named *antimycin*, which is effective as a protectant fungicide in controlling apple scab. But neither of these two substances, fradycin or antimycin, seems to be effective against man's pathogenic fungi. A number of fungicides were isolated from plants. Thomas Sproston and associates extracted such a substance from *Cucumis*, *Tropaeolum* and other plants, while Boris Sokoloff isolated a fungicide (*tillandsin*) from Spanish moss, but none of these substances seem to have therapeutic value in the treatment of human fungal diseases.

This important field is still only slightly touched on by investigators of antibiotics.

11

Actinomycin and Streptothricin

ONCE SCIENTISTS had nothing but curses for the lowly molds that alighted on their agar plates. Today in the bacteriological laboratories the situation is quite different. A mold-contaminated plate is treated royally. It is pampered and catered to, fed the best food, examined and tested. Every scientist hopes that by luck, perhaps another penicillin bearing mold may fall into his hands. The chances, strictly speaking, are slender. The odds are a thousand to one, or perhaps even greater. But there is a pleasant fascination to this pastime.

The Oxford workers have long been excited by this pursuit, and like hundreds of their colleagues are still on the trail of unknown therapeutic molds. Once during a routine inspection of some agar plates they found a contaminating colony of mold. The brown discoloration of the medium attracted the attention of Drs. Chain and Gardner, pioneers in the field of antibiotics. They analyzed the mold thoroughly, subjecting it to vigorous tests. The captured mold exerted a strong germ killing effect in the test tube and looked like an actinomyces, a mold bacterium. They classified it as belonging to this group. It grew rapidly at a temperature of

24° C and much more slowly at a higher temperature, seeming definitely to prefer a cool environment

Experienced as they were the British scientists had no difficulty in extracting the substance that destroyed the germs. It was a white powder, easily soluble in water, and much more stable than penicillin. The yield from one liter of the culture fluid in which this organism grew was almost three times greater than the yield from the best culture of *Penicillium*. The mold was apparently a much more active manufacturer of its drug than the famous Fleming mold. The drug itself—called *proactinomycin*—killed streptococci in a dilution as low as 1 : 1 500 000. These facts were promising. But the tests on animals were a great disappointment. Five milligrams of the substance was sufficient to kill a mouse almost immediately. The new drug was so toxic that clinical investigations were not even attempted. The Oxford scientists recognized that it could hardly be utilized for human needs¹.

Dr Waksman, now famous for his discovery of streptomycin, more than once had similar experiences. One day he and Dr H. B. Woodruff came across an actinomycete, a mold bacterium which proved strongly antagonistic to germs but was of a new species. They called it *Actinomyces antibioticus*, meaning that it affected adversely the normal life of bacteria. When it grew, it did not produce the black brown color characteristic of some mold bacteria but remained a faint yellow in color. This microorganism manufactured not one germ-destroying drug but two and perhaps more substances each exercising a deadly effect upon microbes. One of them merely impeded the multiplication of certain germs; the second was capable of destroying them directly in a manner similar to some antiseptics.

Actinomycin A was an orange pigment soluble in ether and alcohol. *Actinomycin B* was a colorless substance in

soluble in water. In the test tube actinomycin A was bacteriostatic, not killing the germs directly but inhibiting their growth. Actinomycin B was definitely bactericidal and killed the germs directly. However, for all their qualities as powerful germ killers both drugs have been proven quite harmful to animal organisms. Their toxicity is so great that no clinical investigation was ever initiated. They remain testimony to the obstacles with which the scientist is confronted in searching for a new miracle drug. A tremendous amount of energy and time and money were invested by the scientists at Rutgers before it became clear that the substances they had isolated had no therapeutic value.

To laymen such disappointing experiences might be crushing. To spend two or three years of intensive work only to prove that a discovery is of no practical value seems tragic. But a scientist who honestly believes in the value and progress of research is always prepared for such failure. Indeed, from a scientific point of view it is not a failure. The negative results are also valuable and important. No discovery, whether it brings practical results or not, is ever a loss. Its value lies in the extension of our knowledge of the world of bacteria, which is far from complete.

The story of a Russian scientist will illustrate the experiences a scientific worker may undergo only to arrive at an impasse. Five years after Dr. Fleming discovered penicillin, a woman bacteriologist at the Moscow Institute of Experimental Medicine stumbled on a strange phenomenon. A tube filled with peptone broth and left on the laboratory shelf had turned brown black. Why? asked Dr. M. I. Nakhimovskaja. Normally this tube while containing disease-producing germs remained unchanged in color for days or weeks. Obviously it had been contaminated by a microorganism. Even to the naked eye it was plain that a mold-like organism infected the tube.

Examining the contents more closely, the doctor was amazed to find that the peptone broth was almost completely free of disease producing germs. Somehow the germs had been destroyed, the bottom of the tube was filled with the decomposed bodies of millions of dead germs. Dr. Nakhimovskaya at once suspected that the mold which had invaded the culture medium had killed the germ colony. It took considerable time to support her suspicions. She isolated the invader and subjected it to a careful examination. There was no difficulty in recognizing it as a mold bacteria belonging to the same group that Dr. Waksman had been studying the actinomycetes.

Like Dr. Fleming, the Russian woman scientist had to solve the mystery of the dead germs. Again and again she placed the mold in a test tube where germs were thriving and multiplying, only to be thoroughly convinced that there was no mistake about her first observation. This mold was easily capable of destroying disease producing germs.

Having proved the destructive power of the mold bacteria she reversed her experiment. The second step was to learn if all germs were equally susceptible to this germ killer. It was painstaking and time-consuming work for a single person. She submitted various types of germs, one after the other, to the deadly action of the mold. She found no uniformity in its action. While streptococci and staphylococci died quickly in the presence of her mold bacteria, others like the Gram negative bacilli succumbed slowly, as though they had the power to resist the destructive action of the mold. Also there were many types of germs which seemed completely indifferent to the presence of the mold, and continued to live and thrive.

The Russian bacteriologist no longer doubted that the mold she had captured produced an antibacterial substance. The question that concerned her now was the chemical

nature of the substance Her first thought was that the brownish pigment formed in the tube might be the mysterious germ killing agent Examination of the pigment disclosed that it played an important part in the formation of antioxidant substances Perhaps this pigment causes the death of germs by interfering with their respiration she reflected Her reasoning seemed logical for germs like other living cells need oxygen for their respiration and for the digestion of their food a constant oxidation must go on If something in their medium interferes with their normal consumption of oxygen would they not die of asphyxiation?

Dr Nakhimovskaya soon proved her own assumption to be wrong The substance she had isolated although very effective against some germs in the test tube did not interfere with the consumption of oxygen It behaved like many other antibiotics in fact resembled penicillin Next the Russian scientist decided to investigate the other mold bacteria in order to find another mold which possessed the same or greater capacity to destroy germs It was a gigantic task for there are more than a hundred known species of *Actinomyces* Each mold must be located classified then cultivated and afterward tested for its power to kill or to inhibit germs Nothing daunted the Russian woman scientist did not hesitate to embark upon this lengthy investigation

Together with a colleague another woman bacteriologist, she examined fully eighty species of *Actinomyces* over a period of three years The results of their findings were astonishing and perplexing They disclosed that more than half of the actinomyces they investigated were actually capable of producing some antibacterial substance Forty seven of the eighty species of mold bacteria manufacture substances which inhibit, at least to some degree the growth of disease-producing germs The chemical nature and structure of these substances was never so far as we know investi

gated and determined. Their toxicity to animals was never tested. And the investigation was limited to the testing of these antibiotic agents only in the test tube. This work was published in 1939, long before penicillin was recognized by the medical profession and acclaimed as the most important discovery of the century. The work of Nakhimovskaia went unnoticed by the medical profession as a whole, and by scientists working in the same field. Nowhere was there available a reference to the investigation, or to the bacteriologist or to the work she had performed. But from the brief description of the substances she had isolated from the mold-bacteria, some of them were apparently very similar, if not identical, to the actinomycin of Dr. Waksman.

Thirty-three years ago Drs. Waksman and Curtis described a mold-bacterium which they named *Actinomyces lavendulae*. What became of this mold is not known. In all probability, it was lost somewhere in Dr. Waksman's laboratory. In 1942 this mold, or at least an identical mold, was recaptured by Dr. Kocholaty. The microorganism grows rampant in the soil near trees. When Dr. Waksman investigated the organism in the test tube he was impressed by the fact that the substance produced acted very much like streptomycin. He named the drug *streptothricin* and immediately subjected it to various tests. This substance proved highly active against Gram-negative bacilli. In fact, certain germs, like *Escherichia coli*, *Shigella dysenteriae* and some blood-dissolving streptococci fall easier victims to this drug than to its twin brother, streptomycin.² Amazingly, streptothricin can also destroy some yeasts and molds, and there was hope that it might be used as an antifungal remedy.

Experiments with tubercle bacilli, however, were disappointing. Even from the experiments in vitro it was apparent that streptomycin was much more active against *Mycobacterium tuberculosis*.

The first experiments on animals were quite satisfactory. Even small doses of streptothricin protected mice against infection with *Escherichia coli* and the *shigella* bacteria but, according to H. Robinson and his associates, the drug was unable to give protection against pneumococci.³ There was also some indication that streptothricin might be effective in controlling undulant fever germs which cause contagious abortion in cattle,⁴ and is transmissible to man through the consumption of raw milk. Twenty years earlier, in 1924, Belgian scientists, working at the Pasteur Institute at Brussels, had already called the attention of their colleagues to the peculiar ability of these mold-bacteria to annihilate this type of germ.⁵

While on the one hand the experiments on animals were quite encouraging, they were nevertheless a great disappointment to Dr. Waksman, for streptothricin proved so much more toxic than streptomycin that its possibilities for therapeutic use were discounted. When this drug was introduced into the blood stream of a mouse or rat, it produced a nervous shock, an immediate histamine-like reaction. The animal trembled and had difficulty in breathing. These symptoms would soon subside, but two or three days later the animal would die. Autopsies revealed the harmful effects of the drug on the animal organism: the heart showed symptoms of myocarditis; the kidneys, and often the liver, were damaged. But particularly, the mucous membrane of the intestines suffered from the administration of the drug.⁶

It was out of the question to administer streptothricin to human beings by injection. It might be given by mouth, however.

The chemical nature of this substance has more or less been determined. It has an organic base, low in nitrogen, and is easily soluble in water, but is insoluble in ether or acetone. The drug is very thermostable, much more so than

penicillin.⁷ However, since streptomycin is much less toxic and in some instances more effective than streptothricin, it is probable that the latter may never find its application in practical medicine, and must be termed a "therapeutic" failure.

12

The Story of Tyrothricin

MOLDS PREY on bacteria and destroy them. Yet the conflict in the world of microorganisms is not confined to the single struggle of molds versus microbes. Some harmless bacteria exercise a similarly destructive effect upon bacteria of other kinds among them disease-producing germs. This was demonstrated dramatically by the scientists at the Rockefeller Institute for Medical Research.

Dr. Rene Dubos of the Rockefeller Hospital has a creative mind. In his scientific research he is ever seeking new approaches to medical questions. He does not hesitate to attack the problems of medical science in an unorthodox or even in a revolutionary fashion. Twelve years ago, using a commonly known fact as his point of departure, his research culminated in an important discovery.

It is generally known that the soil eventually decomposes all organic matter admixed with it. If a piece of meat or bread is buried in the soil, in due course it will be decomposed and will disintegrate. Numerous soil bacteria are responsible for this. Though ignorant of the bacteriology of the soil, man working the land has for centuries been aware of this power of the soil. But what happens if disease-producing

bacteria are mixed with the soil? Will they remain alive and multiply? For more than fifty years it has been known that the most virulent bacteria such as typhoid bacilli or some streptococci soon die if they are put into the soil for they are destroyed by the living organisms in it. This may be easily proved by a simple experiment. If soil is heated long enough to sterilize it completely and typhoid or diphtheria bacilli are buried in it they will remain alive and may exist for a long time unharmed and vital

That there were soil bacteria capable of destroying germs was suspected by scientists long before Dr Dubos began his investigation. But his audacious idea was to capture these bacteria and to make of them a drug to fight infectious diseases. In 1937 it was a revolutionary idea. Dr Dubos not only proved that such soil bacteria do exist demonstrating his contention with the skill of a great scientist he also isolated for the treatment of human beings those substances produced by the soil bacteria.

Dr Dubos' experiments deserve to be set down as examples of truly scientific research. He mixed a sample of ordinary soil with calcium and phosphorous salts and to increase the number and vigor of the soil bacteria kept the tube in a warm humid chamber for several weeks. He then put a small amount of this enriched soil into tubes swarming with deadly germs the special type of staphylococcus which produces one of the forms of pneumonia. When after two days he finally examined the tubes almost all of the deadly germs had been destroyed their bodies in a partially or completely decomposed state. There was no doubt that the soil bacteria had exercised this devastation on the aggressive microbes. During two years of careful and patient investigation Dr Dubos repeated his experiments again and again using various combinations of salts and modifying the time and the temperature. In 1939 he was ready to report his astonishing

results. He had succeeded in isolating the substance produced by the soil bacteria which was destructive to the disease producing germs. This substance was water insoluble, but was soluble in alcohol. Experiments in the test tube fully demonstrated that it possessed highly bactericidal properties against Gram positive microorganisms. The bacterium from which this substance was obtained was named *Bacillus brevis*. It is a motile sporeforming bacterium belonging to the genus *Tyrothrix*. The drug was first called *gramicidin*.

When the substance was more closely investigated and purified, Dr. Dubos and his associates discovered that it was actually composed of two different substances. The most active and therefore the most important from the medical viewpoint retained the name of *gramicidin*. Its chemical structure was that of a polypeptide and contains no free amino or carboxyl groups.¹ It proved effective against Gram positive microorganisms but had no power to destroy the Gram negative germs.

The second substance was named *tyrocidine*. It was also a polypeptide but it contained free amino groups. Tyrocidine had some power against Gram negative microorganisms such as *H. influenzae*. Consequently Dr. Dubos decided to change the name of the mixture of these two substances to *tyrothricin*.

When the true nature and composition of tyrothricin was established, an extensive investigation of its property to destroy various germs was begun. Herrell and Heilman were the first to show that even a very diluted solution of this drug was able to inhibit the multiplication of pneumococci and streptococci.² When the drug was applied in a weak dilution as a mouth disinfectant it worked as effectively as 95% alcohol.³ The most striking fact, however, was the power of this drug to destroy in the test tube such unicellular organ-

isms as *Trichomonas vaginalis* which quite often causes inflammation of the vagina

The next step was experimentation on animals. Would tyrothricin be as efficient here as in the test tube? That was the crucial moment for Dr. Dubos' investigation. The first investigation appeared to give satisfactory and even encouraging results. When mice were infected with large doses of virulent pneumococci and simultaneously received the drug the fatal infection was prevented. Moreover mice which already showed the deadly effect of the germs and the first symptoms of infection were cured with tyrothricin. However, even from the first attempts to cure or to prevent the infection in animals it was evident that this drug too was highly toxic when injected into the blood stream. It was a great disappointment to many scientists who had followed the work of Dr. Dubos with keenest interest. For the acid test of any drug of this kind is its toxicity. The drug may perform miracles of germ destruction in the test tube but if it is harmful to the cells of the human organism its therapeutic value will be small indeed. Tyrothricin is typical of those antibiotics which were effective and promising in the test tube but when introduced into the blood stream were found to be harmful to the organism. For it destroyed not only the germs but the red blood cells as well. When tyrothricin is mixed with blood the red blood cells disintegrate. Not only was tyrothricin highly hemolytic but if introduced into the system by injection it could cause grave damage to the liver, the kidneys or even to the nervous system. In fact it is harmful to all types of cells including spermatozoa. Only very small doses of this drug could be given to animals without the danger of killing them.⁴

Some investigators attribute this toxic effect to the tyrocidine which comprises one of the drug's ingredients. Dr.

Dubos himself is inclined to believe that gramicidin the principal compound of the drug is less toxic than the mixture.⁶ As the result of these experimentations no clinical investigations with tyrothricin by means of intramuscular injections were practicable. Consequently, this drug is used only for topical applications for the treatment of local infections of the skin or eye or in sinus infections. Even then, the drug should be used in very diluted form in order to avoid possible irritation of the skin or the mucous membrane. It is marketed in the form of an alcoholic solution.

At present, tyrothricin is also being used by surgeons and physicians for local applications. If a surgical wound does not heal properly the doctor may use a weak solution (0.5 per cent) of this drug. Or an ointment containing a small amount of tyrothricin may be applied to the infected area. Such an ointment has considerable healing property on infected burns. During the war some of the Army men serving in the tropics got *pyoderma ulcerosum tropicum* a persistent ulceration of the skin resulting from infection by hostile microorganisms. Tyrothricin has been proved valuable in the treatment of this infection. Surgeons prescribed wet packs of the drug to be applied to infected surfaces.⁶

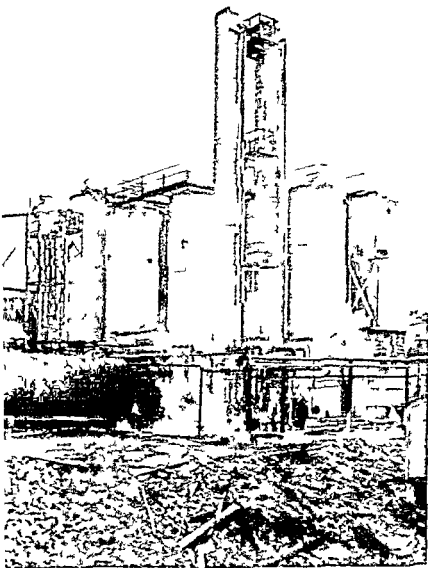
When the mucous membrane of the eye is inflamed with conjunctivitis the doctor may prescribe drops which contain 3 per cent of tyrothricin. This drug has also been reported helpful in chronic conjunctivitis and dacryocystitis.⁷ However, it must be used with caution because medications containing more than 3 per cent may cause irritation of the membrane and thus aggravate the initial infection.

Sinus infections are very common in this country particularly in the large cities of the East and Middle West where sudden changes in temperature and drafts in subways and buildings are encountered. It is estimated that about 20 per cent of the population of New York and Chicago suffer



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FILTERING AND SUBDIVIDING CONCENTRATED SOLUTIONS OF STREPTOMYCIN



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BACITRACIN PLANT TERRE HAUTE, INDIANA

from some form of sinus infection, ranging from a mild form of sinusitis, which may exist for years without being recognized, to a grave condition which requires surgical intervention and long treatment. No wonder every promising antibacterial drug is tried by the specialists. Many doctors have tried tyrothricin in various types of sinus or ear infections. Again, owing to the toxicity of the drug, the results have not been completely satisfactory. J. E. Bordley and his associates and S. J. Crowe have reported excellent results in postoperative treatment of sinus and ear infections due to streptococci and staphylococci.⁸ But grave complications also resulted, due to the drug's toxicity.

A man entered Johns Hopkins Hospital in Baltimore with an acute infection of the frontal sinus, the sinus was irrigated with tyrothricin. The result was disastrous. Almost immediately, the patient developed a high fever, became delirious and complained of severe headache. Twelve hours later it was evident that meningitis had developed. For seven days the patient suffered a grave inflammation of the brain from which he recovered, although not completely. He left the hospital unable to see clearly with his right eye, and suffering some difficulty with his left. The same hospital reported another similar case.

Although these cases are exceptional and Drs. F. J. Otenasek and D. Fairman did not find any other report of ill effects in human beings from clinical use of tyrothricin, the toxicity of this drug must always be kept in mind by the physician who prescribes it to the patient in specific instances. "Even extremely small amounts of tyrothricin, in the cerebrospinal fluid may produce disastrous effects," these doctors warned. "When an opening of the dura mater is known to exist after a radical sinusotomy, irrigations with this agent should be scrupulously avoided."⁹ In layman's language, when a sinus operation has been performed and

there is an open communication with the brain cavity, no tyrothricin should be used

The story of tyrothricin, which developed so dramatically, and looked so promising in the test tube, helps to point up the great value of penicillin. Without the low toxicity factor of penicillin, it could never have been applied so widely. To repeat the essential premise for any 'miracle drug' is its low toxicity, its complete harmlessness to human beings. In this respect tyrothricin did not fulfill early expectations.

Shortly after Dr. Dubos succeeded in isolating tyrothricin from his *Bacillus brevis*, the Soviet medical press announced that the scientists working at the Moscow Institute of Microbiology had discovered a similar substance. A Russian physician, Maria Brazhnikova, disciple of the famous bacteriologist, Professor Alexander Taracevitch followed the same pattern of investigation as Dr. Dubos. She and her associate Dr. G. F. Gause, were able to isolate from the soil a *Bacillus brevis*, similar but not identical to that of Dr. Dubos.¹⁰ They produced a drug in crystalline form known as *gramicidin S*, the letter 'S' for Soviet. It is often called 'Soviet gramicidin'.¹¹ In the test tube it worked similarly to Dr. Dubos' gramicidin. It is, however, somewhat more powerful against staphylococci than the regular gramicidin, but less so against streptococci and pneumococci. The Russian drug is less toxic than American gramicidin yet not sufficiently harmless to be used for injections into the bloodstream. Consequently it is used largely, if not exclusively, for local application, such as the treatment of wounds, eczema and sinus, ear, and eye infections. During the war this drug found considerable use in the treatment of gun wounds when applied in an ointment or in wet packs. Like American tyrothricin, Soviet gramicidin has its therapeutic limitations, owing to its toxicity to the human organism.

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Bacitracin—Subtilin and Polymyxin

DR FRANK MELENEY, head of the Department of Surgery, at the Presbyterian Hospital, Columbia University Medical School, is, in addition to being a first rate surgeon, a brilliant scientist. Following the discovery of penicillin, an extensive investigation of antibiotics was carried on in his department. In fact, his laboratory is among those outstanding in this field.

It was common knowledge that some harmless bacteria present in the soil or water are capable of producing antibacterial substances. But could germs which are themselves the causes of infection exercise an inhibiting or destructive effect upon other disease producing germs? That was the question which interested Dr. Meleney. Often, when there is an infected wound, several types of bacteria flourish in the damaged and inflamed tissue. Is there a war among these germs? In some infected wounds the dangerous streptococci seem to disappear completely and are replaced by relatively harmless bacteria. Perhaps these bacteria have a weapon of their own, a drug which kills the aggressive streptococci. These questions had been in the mind of the surgeon for a long time, but he had not as yet been able to capture any

germ which would effectively fight the more aggressive and dangerous bacteria

At this time seven year old Margaret Tracey fell and broke her leg while playing with her friends. The tibia the large inner bone of the leg was fractured. Recovery was slow and an infection set in around the damaged tissue. She was operated on by Dr. Meleney. In the debrided tissue removed from Margaret's fractured leg he discovered an organism later identified as *Bacillus subtilis*. He prepared a crude filtrate from the culture of this bacterium which was found to inhibit the growth of the deadly blood-dissolving germ hemolytic streptococcus. With the assistance of Dr. Herbert Anker Dr. Meleney extracted from the filtrate a yellowish antibiotic powder.

In honor of the patient from whom the microorganism had been isolated the drug was named *bacitracin*. This substance was found to be of low toxicity and could be introduced into the blood stream of animals without harm. However due to difficulties in purification the drug has been used till now only for local treatment of infected wounds in surgical infections.¹

In his recent report Dr. Meleney summarized his observations on this drug conducted on one hundred cases over a period of about three years. He recounted a number of remarkable case histories.

One patient had a diffuse infection of the finger. *Bacitracin* was injected directly in the infected area and the finger was rapidly cured.

A young man had been suffering from six stubborn ulcerating infections on the legs, arms and buttocks. The examination revealed that the cause was hemolytic streptococcus. Dr. Meleney made a comparative test. Some of these ulcers were treated with penicillin others with *bacitracin*. The results were in favor of the new drug. As Dr. Meleney

stated, 'It was obvious that in every instance the bacitracin treated ulcers lost their exudate and healed faster than did the penicillin treated ones' ²

The son of a professor of bacteriology at one of the Southern medical schools was brought to the hospital with an acute infection around an ingrowing toenail. The portion of the nail and its matrix were excised and the wound left open, and dressed with bacitracin ointment. Two days later the wound was perfectly clean. By the fourth day the wound itself was closed and 'looked as if it were healing by primary union' ³

Perhaps even more remarkable is the case of the patient suffering from a deep abscess of the cheek. Such an infection is always painful and of long duration. A small amount of the drug was injected directly into the infected tissue on two successive days. By the fourth day, the swelling was all gone and the need for surgical intervention was obviated.

While remarkably effective in some infections bacitracin seems of little value in others. One patient had numerous furuncles of the axilla. The drug was injected into the boils but produced no curative effect. The germ which caused the infection was identified as *Escherichia coli*, a bacillus which seems to be resistant to this drug. Similar discouraging results were observed in the treatment of a patient with multiple furuncles of the face. Local application of bacitracin ointment did not bring any relief. However, penicillin caused a prompt disappearance of the boils.

There are some indications that this new drug which is still being investigated, may be as effective as penicillin, at least in local treatments of infections caused by Gram positive organisms. It appears that bacitracin is much less active against staphylococci and nonhemolytic streptococci than penicillin, but is highly potent against hemolytic streptococci, and pneumococci.

Animal experiments have shown that bacitracin gives some protection against germs of the group *Clostridia*. In all probability the drug, when available in pure form, will find its application in some infections where penicillin is not sufficiently efficacious or when bacteria are encountered which are resistant to penicillin.

Paradoxically, bacitracin is another product of a simultaneous discovery by two different groups of scientists. As with the cases of patulin and clavacin, the discovery of a drug similar, if not identical to, bacitracin was announced by other scientists almost at the same time that Dr. Meleney isolated the substance from the fractured leg of his patient. In 1944, Drs. E. F. Jansen and D. J. Hirschmann, extracted an antibiotic substance from *Bacillus subtilis*. They called it *subtilin*, and gave a detailed description of its physical and chemical properties.⁴

However, to tell the complete story of this discovery it is necessary to turn back to 1925, when Dr. L. Rosenthal, while working at Pasteur Institute in Paris, found that a certain microorganism possessed a remarkable activity against disease-producing germs. At that time he identified this organism as *Tyrothrix scaber*.⁵ For a number of years no report of this discovery was made. But when interest in antibiotics began to widen, the work of Dr. Rosenthal was remembered and he himself harkened back to his twenty-year-old discovery. He soon found out that the organism had been wrongly classified and was actually nothing but *Bacillus subtilis*, the same bacillus from which Dr. Meleney extracted the new drug, bacitracin. In his more recent investigations, Dr. Rosenthal disclosed that many strains of *Bacillus subtilis* produce substances which destroy certain types of pathogenic germs.

Shortly after the announcement of Dr. Rosenthal's rediscovery the workers at the Western Regional Laboratory, United States Department of Agriculture, Albany, California, began

the investigation of this bacillus, known otherwise as Hay bacillus, and succeeded in isolating the drug, subtilin. The substance isolated by Drs. Jansen and Hirschmann was a crystalline product, apparently of a polypeptide nature, soluble in alcohol and inactivated by light. The drug is highly active against *Staphylococcus aureus* and *Staphylococcus viridans*, but not against *Bacillus typhosus*. A number of disease-producing fungi were also found to be susceptible to subtilin. The drug produces an inhibiting effect upon the bacteria in high dilution. When tried in stronger concentration, it destroys the germ directly. Thus it is both bacteriostatic and bactericidal.⁶

Dr. Hamilton B. Anderson and his associates have shown that the drug is slightly hemolytic, but less so than gramicidin. It is of relatively low toxicity, particularly when given to an animal by mouth. According to these investigators it possesses a remarkable activity in the test tube against the parasitic amoeba, *E. histolytica*. One monkey was freed of this parasite after a ten-day treatment with subtilin.⁷

It has not yet been determined whether bacitracin and subtilin are identical substances.

Antibiotics are being developed at a bewildering rate. In this country and abroad thousands of scientists are engaged in the search. In the laboratories of commercial companies, in the bacteriological laboratories of medical schools, in the hospitals and medical institutions, almost everywhere molds and bacteria are painstakingly investigated. Since the isolation and examination of an antibiotic isolated from a mold or bacterium often takes a year or more, it is not surprising that the same mold and the same drug may be discovered in two, or even more laboratories at the same time. Perhaps a bacteriologist at the Pasteur Institute in Paris has been able to isolate the same mold or bacterium that a worker at Rutgers University is testing for therapeutic purposes. For a

long time to come scientists themselves as well as laymen will be bewildered by a variety of labels for the same substance

Among the numerous antibiotic substances isolated and described during the last two years one of the most promising seems to be *polymyxin*. This drug was obtained from the culture medium of *Bacillus polymyxa* commonly found in soil and water. Drs. R. G. Benedict and F. H. Stodola of the Northern Regional Research Laboratory, Peoria, Illinois, discovered this substance and demonstrated its antibacterial activity against a group of the most stubborn germs susceptible to neither penicillin nor to streptomycin. *Escherichia coli*.⁸ There is evidence that this drug may be effective against many other Gram negative organisms such as *Bacillus pertussis* the germ which causes whooping cough or the bacteria responsible for intestinal infections.

Whooping cough is far more dangerous and widespread than is generally recognized for many children succumb to it. Children under three are often the victims. The infection is transmitted by contact with a child who has the disease or by a healthy carrier of the germ. No effective remedy is yet known therefore any drug capable of combating this infection will be enthusiastically received.

Recently Dr. Emanuel Schroenbach and his associates at the Johns Hopkins School of Medicine reported seven treated cases of whooping cough with polymyxin among them a six week old baby and his thirteen month-old brother who were seriously ill. The temperature of the younger baby had reached 103° when the drug was first given. Within one day after polymyxin was started his temperature returned to normal. The physicians are as yet reluctant to draw conclusions from their investigations. But they have been en-

couraged by the early reactions for apparently the lives of the infants were saved by this drug

Polymyxin seems to be of low toxicity and can be given to man either by intramuscular injection or by mouth To date its use with only a limited number of diseases has been reported Besides whooping cough and Friedlander's meningitis, polymyxin is effective in bacillary dysentery Dr Sidney Ross and associates of the Children's Hospital Washington, D C, reported the results of the treatment of 16 children affected with bacillary dysentery (*Shigella* a causative agent) with this drug In two days the germ disappeared from the stool of the majority of patients, and recovery took place And yet in a few of the treated children the bacillus was still found after several days of treatment⁹

Although the Gram negative germs, like bacillus dysentery, do not cause such serious diseases as for example, do streptococcus and staphylococcus which are both Gram positive bacteria, when they do cause an infection it is generally stubborn and dangerous The necessity of a drug an antibiotic, effective against this type of infection has been recognized since the time that the limitations of penicillin in this respect were realized The search directed along these lines has been very fruitful Three new antibiotics were recently discovered which seem to answer this demand All three already have established their therapeutic value and are being used on a large scale They are aureomycin, terramycin, and chloromycetin, otherwise known as chloramphenicol *

* Chloromycetin is the trade name for Chloramphenicol manufactured by Parke Davis Co

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Aureomycin, Chloromycetin, and Terramycin

THERE ARE hundreds of mysterious disease-producing agents which belong to the group of "filtrable virus." Mumps, measles, chickenpox, and influenza are only a few of the virus infections which attack men and animals. Some cause such dangerous diseases as poliomyelitis, epidemic encephalitis, rabies, and yellow fever. Indeed, virus diseases range from the most dangerous and fatal infections to completely harmless conditions. Some produce devastating effects upon the human organism, and they may harm or destroy vital organs. Other viruses produce no ill effects and, in fact, may not even cause clinical symptoms.

What is the nature of viruses? Why are they called "filtrable"? How do they differ from bacteria? Viruses are so minute as to be invisible. Therefore, they pass freely through the pores of a filter. They cannot live and propagate without living cells. They penetrate the cells of their host and, on living cells which are susceptible to them, produce a destructive effect, causing lesions and deformations where they invade. This is the fundamental difference between viruses

and bacteria, in most cases the latter have no close relation to the cells of the organism they attack.

Are the viruses living organisms? Some scientists have expressed doubts, for all their characteristics are manifested only when they are in association with living cells. Actually, they possess all the fundamental attributes of living substances. They assimilate and metabolize nutritive substances. They multiply and reproduce. And they are able to adapt themselves to environmental changes. They have chemical specificity and, if submitted to the influence of chemical and physical factors, respond as promptly as other living substances.

Viruses are minute indeed, and are often called 'the midgets of the microbial world'. However, they vary in size. Among the smallest is the virus of polio, which is about twenty times smaller than the virus of parrot fever. The virus of yellow fever is slightly larger than the polio virus.

But it is not their size that makes the fight against them so difficult and often so hopeless. It is their close association with the cells of the organism which makes them dangerously resistant to medical measures. They invade the cells of the body, and almost immediately adapt themselves to the chemical environment of the tissue fluid. Figuratively speaking, they make themselves perfectly at home. To a large extent they become part of the cell, and the cell is diseased and partially disorganized by them. The reason the fight against the infectious diseases caused by viruses has not been successful is because the therapeutic drugs must have the unique property of destroying the viruses in the cells, without damaging the cells themselves.

All the antibiotics so far discovered have shown little effectiveness against viruses. Penicillin, which can conquer many disease-producing bacteria, is powerless against viruses. Penicillin was tried repeatedly, *in vitro* and *in vivo* against

various types of virus only to bring negative results. In some cases like that of the virus of parrot fever, penicillin has produced some results in animal experiments but not sufficiently clear and definite to afford the hope that the drug may be used for the treatment of virus infection. Streptomycin, patulin, bacitracin, tyrothricin, streptothricin—none has proved capable of destroying virus.

Yet there must be some antibiotic which will succeed against viruses. The efforts of scientists at present are bent in this direction. Dr. S. Waksman feels that to find a drug against viruses is the most urgent problem of the science of antibiotics today.

If a drug could be found which would be as effective against viruses as penicillin is against the Gram positive bacteria, then the struggle against the disease producing germs will enter its last victorious stage. In this respect three new drugs, aureomycin, chloromycetin, and terramycin show some although slight anti virus activity.

AUREOMYCIN

After testing many substances produced by the mold bacteria *Streptomyces*, Drs. B. M. Duggar and C. W. Hessel, of Lederle Laboratories, came across a mold named *Streptomyces aureofaciens* and succeeded in isolating a new substance which possessed unusual antibiotic properties. The antibiotic was named aureomycin for its red-gold color. Animal tests revealed a striking spectrum activity. The drug exhibits a definite activity against many rickettsiae and certain viruses. Most important, it is of low toxicity to man.

Often the tragedy of the past is easily forgotten. The names of the scientists who lost their lives serving the cause of humanity are hardly known, even to those who work in the same field. The name rickettsia, as applied to the infectious disease known as Rocky Mountain spotted fever, may

be familiar to a few. But who remembers the origin of the word "Rickettsia"? Forty years ago this name was linked with dramatic events. In 1909 an American scientist, A. Ricketts described a microorganism which he had discovered in the blood of patients with Rocky Mountain spotted fever. The next year, together with Dr. Wilder, he found similar organisms in the blood of typhus fever patients. Dr. Ricketts plunged deeply into an investigation and soon was able to prove that lice transmitted the disease from sick persons to healthy ones. He found the same organism in smears from lice as he found in the infected patients. While working on this problem, and trying to save patients affected with Rocky Mountain spotted fever, Dr. Ricketts caught the infection which ended in his death.

Almost at the same time, a famous German protozoologist, S. von Prowazek, made a similar discovery while investigating an epidemic of typhus fever in Serbia. He, too, contracted the infection and like his American colleague, died a victim of his devotion to science. A few years later the germ was named *Rickettsia prowazeki*, in honor of these two scientists. It is the only monument to their heroism.

The rickettsiae are not viruses. They belong to the bacteria class, but they have biologic qualities in common with the viruses for they have a close relationship with the cells of the host. Like the viruses, they have never been cultivated without the presence of living cells. The only growth of these organisms that has been obtained outside the animal body has been in tissue cultures.¹ They need the presence of living cells to multiply, and as a base for their aggressive existence. Perhaps due to this characteristic, rickettsiae have so far shown remarkable resistance to antibiotic substances. However, animal experimentation indicates that these organisms are quite susceptible to aureomycin.

A case of typhus fever treated and promptly cured by aureo

mycin was recently reported by Dr Emanuel B Schoenbach of Johns Hopkins University Medical School.² Typhus fever is assumed to be caused by a virus organism, transmitted either by a louse or a flea. In this particular case the patient was affected with so-called Brill's type of typhus fever. In all probability he had been some years ago, a victim of epidemic typhus and the present infection was no more than a recrudescence of the old latent disease. It was a relatively mild infection.

A white man Rumanian born was admitted to the hospital with a fever, shaking chill, severe headache and pain in the calves of both legs. His symptoms became progressively worse with a rash appearing on the abdomen. Treatment with penicillin gave him no relief. His temperature rose to 105° F., with the rash becoming more extensive. He was transferred to the Sinai Hospital where administration of aureomycin was prescribed. The treatment was 200 mg. by mouth every hour for three initial doses and, thereafter 200 mg. every two hours day and night. Prompt improvement was noted in the well being of the patient, the doctor states, with diminution of the headache within twelve hours after aureomycin therapy was started. The next afternoon the temperature was almost normal. Three days later he was discharged from the hospital and soon was able to perform his duties as manager of a large supermarket. He had received a total of 6.6 grams of aureomycin orally during the four days of treatment without any apparent ill-effect.

Dr M. Finland and associates reported rapid improvement in some of their patients affected with influenza A and B and a favorable response to aureomycin therapy in many patients with acute respiratory diseases originating in the common cold.³ Even virus pneumonia seems to respond to this drug. Dr Yale Kneeland and his associates at the College of Physicians and Surgeons, Columbia University reported that aure

omycin therapy brought relief to patients affected with virus pneumonia in forty-eight hours

No less encouraging results were obtained in the treatment of so-called atypical pneumonia Dr Schoenbach and Morton S Bryer of Johns Hopkins University School of Medicine have recently reported eighteen cases of atypical pneumonia treated with aureomycin ⁴

This infection of the lungs has been recorded as increasingly frequent during the past fifteen years Unfortunately, the germ which causes this condition is not as yet isolated and little is known about it The onset of this type of pneumonia is usually gradual with fever, headache and chilly sensations Physical examination often reveals only minimal and insignificant signs in the chest which are out of proportion to the roentgen evidence of lung involvement Neither penicillin nor sulfa compounds are of help in atypical pneumonia and the effectiveness of aureomycin is therefore of the utmost importance

Dr Schoenbach tells us about the case of a young woman, 31 years old who was admitted to the hospital with the symptoms of this disease Her temperature was as high as 104° F with a very fast pulse cough and headache She had been ill for five days prior to her admission Immediately aureomycin was prescribed in a dose of 3 grams per day by mouth Within twenty four hours the temperature had fallen progressively to 99.8° F The signs of improvement were evident within twelve hours after therapy was begun After four days of treatment the woman was completely cured of the infection

The doctors concluded that the results have been sufficiently encouraging to indicate that this drug is an effective chemotherapeutic agent for this disease

The activity of aureomycin against some virus infections was further demonstrated by the investigations conducted at

Harlem City Hospital of New York Here twenty five patients with a virus infection known as *Lymphogranuloma venereum* were treated with aureomycin This is a venereal disease identical with tropical bubo which occurs mostly in Negroes male and female The lymph glands in the groin are enlarged and infected and sometimes the rectum is also affected It was Levaditi of the Pasteur Institute who identified this germ as a filtrable virus The germ is pathogenic, not only for men but for monkeys and mice as well Dr Louis T Wright and his associates at Harlem City Hospital were very much impressed with the results particularly because as they stated effective treatment has not been available for this disease *

What were the effects of the aureomycin therapy on the course of this infection? Eight patients with buboes showed a decided reduction in the size of the node at the end of four days treatment Within forty-eight hours after the start of the treatment specimens from the same buboes revealed only occasional traces of the infection The doctors concluded that the drug does have virus-destroying properties that is it is *virucidal*

Aureomycin was also tested extensively on infections of the eye Drs A E Braley and M Sanders treated one hundred patients who had various ocular infections and got very satisfactory results * They concluded that aureomycin (aureomycin borate of 0.5 to 1 per cent solution) is nonirritating to the inflamed mucous membrane of the eye They found the drug effective against staphylococci and pneumococci and it was highly effective in influenzal infections of the eye.

As was said before streptomycin is quite effective in the treatment of tularemia an infection which is transmitted to man from wild rabbits The recent report by Dr T E Woodward and associates of the University of Maryland School of Medicine revealed that aureomycin is even more effective than streptomycin against *B. tularensis* the germ of tularemia

Their conclusion is based not only on clinical observation but is substantiated by experiments on animals.⁷

When mice were infected with *B. tularensis*, and given only 3 mg of aureomycin for four days they all survived, while the other infected mice treated with streptomycin all died. However, when a larger dose of the germ was injected both groups of animals, treated respectively with streptomycin and aureomycin, died almost at the same time (16 days average). This observation seems to indicate that aureomycin is somewhat more active than streptomycin against *B. tularensis*.

The doctors have also treated three cases of tularemia with the new drug. "The response of the patients to aureomycin therapy," they stated, "is regarded as comparable to streptomycin therapy in degree of effectiveness."

Enormous clinical material has been accumulated on the therapeutic value of aureomycin during the last three years. This clinical data is very impressive. Although this drug exerts its most striking effect on infections relatively uncommon in this country (typhoid fever, psittacosis, the rickettsial infections, tularemia) its value in many respiratory infections is beyond any doubt. It is useful and highly effective in the types of pneumonia where penicillin is inactive (atypical pneumonia). The results in peritonitis and some colon-group infections are most gratifying. It is ineffective in virus intestinal infections. It might be of some help in poliomyelitis according to Dr. E. Weis and Dr. B. J. Winston.⁸ They treated 48 cases of acute polio with large doses of aureomycin. The patients who received a daily dose of 2 gm of this drug 87.5 per cent had recovered completely by the time they left the hospital. The duration of fever was about one half as long as in those who were not treated. So far no confirmation of the effectiveness of aureomycin in acute polio has been received from other medical sources. But even if aureomycin is only partially effective in reducing the gravity of poliomyelitis

this would be of prime importance to numerous mothers living in fear of this infection for their children.

CHLOROMYCETIN

Dr Paul Burkholder, of Yale University, has been an ardent seeker of antibiotics. Not satisfied with the mold and mold bacteria he was able to find in this country, he asked for samples of the soil from various countries all over the world. Perhaps, he reasoned, some foreign soil may harbor an unusual drug producing organism. Hundreds of soil samples were sent to his laboratory and promptly investigated. But only one yielded important results. It was the sample of earth from a mulched field near Caracas, Venezuela. From this foreign soil bacterium the substance named chloromycetin was isolated and obtained in a pure crystalline form.²

Recently, chloromycetin was synthesized by Dr Mildred Rebstock of the Parke, Davis Company, together with Dr Loren M Long and Dr Harvey Troutman. They found that the drug was a derivative of dichloroacetic acid, a chemical which never before was found in naturally existing compounds. (The chemical formula of chloromycetin is D threo-1 para nitrophenyl 2-dichloroacetamide-1, 3 propanediol.)

It is claimed that chloromycetin is effective in the treatment of atypical pneumonia, typhus fever, typhoid fever and undulant fever, but no clinical reports have yet been published.

The fact which impressed the investigators was that this substance has a very wide range of action, inhibiting both Gram positive and Gram negative bacteria. Moreover, it is effective in as high dilution as 0.35 micrograms per cubic centimeter, a higher activity than that of penicillin or streptomycin. In the field of rickettsia and virus diseases the activity of chloromycetin seemed truly remarkable. When the drug was injected into chick embryos it proved potent against various types of rickettsia.

Drs. J. E. Smadel and E. B. Jackson of the Army Medical Center, Washington, D.C., demonstrated this in their investigations.¹⁰ They infected twenty-four seven-day chick embryos with various types of rickettsia germs. Shortly afterward, they injected a small amount of chloromycetin. When the dose was as high as one milligram all the embryos remained alive for fifteen days, for the drug had annihilated all the germs. Twenty-four embryo chicks infected with the same germ acted as the control; they were not treated with the drug, and all died within a few days. Similar results were obtained with parrot fever virus, variola virus, and influenza A virus. Apparently this drug is capable, at least in vitro, of destroying virus germs.

When mice were infected with the germ of scrub typhus, the drug reduced the mortality by 62.5 per cent. It was also found that chloromycetin is rapidly absorbed from the intestinal tract (in contrast with streptomycin) and is apparently nontoxic even when given intravenously in massive doses.¹¹ When the mice were given a large dose of the drug by mouth they were protected against the virus of encephalitis.

After having completed their experiments on embryos and mice, Dr. Smadel and his colleagues embarked on a test on human beings. At first they made a preliminary test to find out whether the drug was harmful. They called for volunteers among physicians to serve as human guinea pigs. They also wanted to know how long the drug remained in the blood. When one gram of the drug was given by mouth, it disappeared completely from the blood at the end of eight hours. But when a minimal dose of one gram was followed by 0.2 gram every four hours, it remained in the blood at a level sufficient to destroy the germs. None of the physicians who served as guinea pigs for this peculiar test experienced harmful effects, either during the treatment or after.

Having proved in such a convincing manner that chloromycetin is not harmful to human beings, Dr. Smadel then

result the incidence of streptococcal infections was greatly diminished. Only on rare occasions would this germ invade the wounds. But other germs resistant or indifferent to the action of sulfa drugs had also infected the wounds. Again the doctors were confronted with difficult problems. This time it was the staphylococci which attacked the tissues and blood streams of the patients. Now they had to seek a drug which could effectively protect the wounds from the pus-forming germs. They found the answer in a drug called *proflavin*.

Very little was known at that time about proflavin even among medical men. Yet the few clinical tests made at Glasgow and London Universities suggested that this drug might be effective against some types of germs. The doctors at the General Scottish Hospital decided to try it on their own cases. At first they applied it in solution, that was completely ineffective. Then they sprayed the wounds with a powdered form of the drug, now the results were gratifying. In most cases the wounds remained sterile and healing proceeded with remarkable swiftness.

Strangely enough this antibacterial drug was discovered and described in 1920 by Dr. C. H. Browning of Glasgow University, but did not receive recognition for almost twenty years.

In the search for an antibacterial substance Dr. Browning took into consideration the numerous correlating forces of the organism which participate in the fight against invading bacteria. He conjectured that there must be a reason for the fact that pathogenic bacteria actually live in the human organism for months, if not years, in a quiescent state without attempting to invade the surrounding tissues. Some forces restrain them.

Dr. Browning wondered whether there might not exist

natural antiseptic substances which impede the growth of these germs. He and his associate, Dr. Adrien Albert, undertook an intensive investigation of the protein substances which possess antibacterial properties and are present in the human organism.

The amino group, the building block of the protein molecule, became the point of departure for their investigations. They came to the conclusion that the diaminoacridines were the substances for which they were searching. According to Browning, these natural antiseptics are characterized by three properties. In extreme dilution they act adversely toward a number of pathogenic bacteria, and their action is selective in that animal tissues are relatively unharmed, second, their antiseptic action is undiminished and in some cases even increased, in the presence of blood serum, and third, they are not damaging to the white cells, the defenders of the human organism.

Proflavin does not kill the germs directly. It asserts its effect in a rather complicated physiological manner. One might say that proflavin surrounds the bacteria with a sort of "chemical vacuum." It destroys by depriving them of oxygen, without which most of these microorganisms are unable to live and grow. This drug causes a sort of asphyxiation. The chemical composition of proflavin, and its antibacterial behavior is similar, if not identical, to that of some of the antibiotics.

Thus the three different lines of investigation, that of substances naturally present in the organism, protein, that of synthetic sulfa compounds, and that of antibiotics isolated from molds and bacteria actually work in the same direction, although from different angles. When all the discovered antibiotic substances are compared, whatever their origin, there is considerable similarity in the chemical and biological

properties of some of them. There are others the biological actions of which are similar but which are of a quite different chemical nature.

HOW DO THE ANTIBIOTICS ACT?

The fact which impresses everyone who works in the field of antibiotics is the great variety of these compounds chemically speaking. The antibacterial activity of sulfa compounds it is believed depends on the formation of a free amino group when the compounds are introduced into the body of animal or man. Proflavin belongs to the diaminoacridines; it is possible that the activity of this compound depends greatly on its amino group. Penicillin is assumed to be a peptide composed of two simple amino acids. Gramicidin, tyrocidin and actinomycetin are polypeptides composed of various amino acids. Streptomycin is an organic nitrogenous base (a hydroxylated base containing a free carbon group and a methyl amino group). But on the other hand there are the antibiotics which are of lipid nature like clavacin and pyocyanase. Some are sulfur bearing compounds like gliotoxin and others are pigments like actinomycin A.¹ Exactly which chemical group is responsible for highly antibacterial activity is not yet resolved. It is still a matter for speculation and conjecture. Extensive investigations and experimentations on animals and in the test tube are being conducted. But one fact seems to be quite evident: the most active antibiotics are highly water soluble. Penicillin and streptomycin, both outstanding antibacterial substances, are water soluble. The same is true about the sulfa compounds. As P. Lawrence reported, the more water soluble compounds are the more active.² The newly discovered aureomycin, a very active antibiotic, is also easily dissolved in water.

In what manner do the antibiotics exert their destructive activity upon bacteria? This question is not completely

solved either, and there is much discussion on the "how" of antibiotic activity. Apparently some of these drugs act directly by "dissolving" the germs. Such an action is called *lysis*. These substances are designated as *bactericidal*. However, when the drug does not act directly, but exerts its activity by inhibiting the growth of germs, it is called *bacteriostatic*. The majority of the antibiotics are bacteriostatic. Yet some of them are capable of acting both ways: to kill the germs directly and to inhibit their growth. We have stated these facts earlier in this work.

The sulfa drugs are, as a rule, bacteriostatic—as are actinomycin, patulin, aureomycin, terramycin, and chloromycetin. Actinomycetin, actinomycin B., chorellin, gramicidin, pyocyanase, and tyrocidine are bactericidal. It is assumed that penicillin, streptomycin, penatin, and clavacin are both bactericidal and bacteriostatic.

How do the antibiotics achieve their purpose? By what means, chemical or physical, do they exert their effect upon the germs? Here again, there is a great variety in their methods. These processes are not simple, nor are they entirely defined and understood. Various theories have been suggested. Generally speaking, the mode of action of an antibiotic consists of interfering with the multiplication of germs, by blocking or disrupting their metabolic processes. Some antibiotics seem to interfere with the respiration of oxygen. Proflavin behaves in this way. Some of the sulfa compounds (sulfathiazole) act in a similar fashion (Fischer and Armstrong). There are antibiotics like subtilin and gramicidin which lower the surface tension of the cell of the bacterium. But the opinion has also been expressed that gramicidin interferes with the proper utilization of amino acids by the bacteria.

There has been much discussion about the action of penicillin. Dr. H. J. Weishimer contends that penicillin deprives

the bacteria of the food they need. In this manner the drug produces its lethal effect on many types of germs without, however, harming human beings. As he explained, one of the products of our metabolism is a substance known as pyruvic acid. Both the living cells of our body, and the germs that invade the body, utilize this substance. For both, pyruvic acid is an essential nutrient, without which they could hardly exist. However, the bacteria and the living tissue of the human organism consume this substance in altogether different ways, so that, while penicillin obstructs the use of pyruvic acid by bacteria, it does not interfere with the normal utilization of this substance by the human body.

Other scientists (Gardner, Chain, and Duthrie) have given quite a different picture of the mechanism of penicillin action. Bacteria pass, as do all living organisms, through a cycle of multiplication which is followed by rest. It is precisely the young bacteria which are so susceptible to penicillin. Growing bacteria require more oxygen than the older generation. In the presence of even small amounts of penicillin the oxygen intake by baby bacteria is completely stopped, while the older bacteria are still capable of using the oxygen so essential to them. It is possible, however, that oxygen is not the only substance of which penicillin deprives them, and that the interference with the proper utilization of pyruvic acid is also an important factor in the destruction of germs.³

Penicillin not only inhibits the growth of the bacteria but also affects them directly by lysis in which the bodies of the bacteria are gradually disintegrated. The mechanism of lysis is not yet properly understood.⁴

What is the principle of the action of sulfa drugs? C. P. Miller and his associates believe that it is similar to that of penicillin.⁵ Some of the sulfa compounds seem to interfere with the consumption of oxygen, but they also make it im-

possible for the bacteria to obtain a vital food substance, without which they cannot grow. Figuratively speaking the sulfa drugs act by surrounding the germs with a sort of chemical magic wall which interferes with the regular supply of essential material. In the presence of sulfa drugs the bacteria are unable to multiply rapidly. In fact, if they are sufficiently susceptible to the drug they do not multiply at all. No longer having the vitality necessary for an offensive war, they lose all their virulence and remain on the defensive, most of them stay alive but are greatly weakened by the lack of food.

What is this vital food substance which is denied the germs by the intervention of the sulfa drugs? What is the element so necessary for the growth of bacteria? The answer was given some time ago by Dr J. M. Gillespie. He pointed out that one of the factors of the vitamin B complex plays an essential part in the growth of some bacteria. If the para aminobenzoic acid is lacking in the nutritive medium the bacteria either do not multiply, or their growth is considerably impeded. This substance is widely distributed in nature. It can be found in small amounts in the blood and tissues of animals and man as well as in many food substances. It is present in considerable quantities in yeast.

As we pointed out earlier, two British scientists, Drs Woods and Fildes, were the first to observe that para aminobenzoic acid exerts an antisulfanilamide action. To mice infected with streptococci they gave doses of sulfa drugs. The infections could not progress. The animals were properly protected against the germs by the drug. But when during the treatment they fed the same mice a large quantity of yeast they saw that the sulfa drugs were less effective. In some strange way this vitamin (para aminobenzoic acid) in the yeast interfered with the sulfa drug action. Why? How does this work?

Apparently para aminobenzoic acid is an element essential

to bacterial life. It plays an important part in bacterial metabolism. When a sufficient amount is present in the medium or in the blood stream, it forms some sort of protective chemical wall against the sulfa drugs and blocks the way. But if only a trace of para-aminobenzoic acid is present, as is usually the case in the human and animal organism, the picture is reversed. Then the sulfa drugs form a chemical wall around the bacteria and block off the road to para-aminobenzoic acid.⁶

TOXICITY

An antibacterial drug must be almost completely harmless, that is a fundamental requirement before it can be applied for therapeutic use. An antibiotic may be very active against germs in the test tube but if it is toxic to the human body, the drug is therapeutically valueless. In this respect, most of the sulfa compounds are toxic to some degree to the human body. Among the several hundred sulfa compounds which have been developed in the last ten years or so only about ten have been adopted for medical use. Even those widely used are far from ideal antibacterial agents. For an ideal drug would be completely harmless to the tissues and the blood forming system of human beings.

On the other hand, up to now one hundred natural antibiotic substances have been isolated. Some are the products of molds and others have been extracted from the bacteria of soil or from other harmless or pathogenic bacteria. A number of substances which seem to possess some antibacterial activity were discovered in plants and even in insects.⁷ But from this mass of antibiotics bearing the most fantastic names there are actually only a few which have been accepted therapeutically. The investigations on the rest never reached the clinical stage. For animal experimentation proved their high toxicity beyond any doubt.

In order to be effective in the blood stream, an antibiotic must remain in the system at a certain level for a relatively long period of time. The more stubborn and acute the infection, the larger the dosage which must be given, and the longer the treatment which is required. Thus, *low toxicity is an imperative prerequisite*. A drug is considered of very low toxicity if as large a dose as 0.5 gram or more per kilogram of weight can be given without harmful effect to the tissues, blood, and organs of the patient. Unfortunately, the only drug which more or less answers this requirement is penicillin. In spite of all the extensive investigations conducted during the last ten years, no other substance of as low toxicity to the organism and yet as powerful against certain germs has been discovered. No other drug has surpassed it in low toxicity, although even penicillin cannot be considered completely harmless. Aureomycin, terramycin and chloromycetin are also of low toxicity and very close to penicillin in this respect. Streptomycin, on the other hand, is of considerable toxicity and should be given, if in large doses and for a long period of time, with caution.

The most important feature of an antibacterial drug's action with regard to toxicity is its effect on the blood. How do the white and red blood cells behave and how do they react to the drug when it enters the blood stream? Before any antibiotic is given to human beings, it is tested for these properties.

It has been known for some years* that sulfonamides are toxic to white blood cells, although the effect is only temporary if the dosage is moderate. However, if the drug is given for too long a period considerable harm may be done to the white-cell producing system.

Sulfur drugs also have a harmful effect upon the red cells. Anemias are quite common in patients treated with this

* See pages 50-51

drug. However, in this respect sulfathiazole is much less toxic than sulfanilamide and sulfapyridine.

The most feared complication of sulfa-drug therapy is the disease of blood formation known as agranulocytosis.⁸ This disease may occur when the drug is given fifteen days or more. The daily dosage is less important than the length of time of the treatment. Agranulocytosis is often the cause of death. The most dangerous drug is sulfapyridine, sulfathiazole is less toxic, the least harmful is sulfadiazine.

Penicillin, in the dosage given to patients, does not produce any damaging effect either on the white cells or on the red cells. It is never the cause of an anemia, and can be given to patients suffering from any type of anemia. Penicillin has no injurious effect upon the blood forming system, a fact of great importance to the medical practice.

Streptomycin does not affect either the white or the red blood cells and does not induce anemia of any type. Chloromycetin is known to cause granulocytopenia and, therefore, might cause agranulocytosis.

Tyrosinemia is highly destructive to red blood cells. In fact, it is so hemolytic that it cannot be introduced into the blood stream at all. On the other hand, bacitracin and aureomycin appear to be harmless to red and white cells.⁹

How does the antibiotic affect the kidneys? That is the second major question asked about any drug which must be given in large doses and often for a long period of time. Sulfathiazole, the sulfa drug commonly prescribed, produces a damaging effect upon the kidney but damage to the kidney is relatively less frequent in sulfanilamide therapy.¹⁰

Penicillin has no effect upon the function of the kidneys. It can be given freely even to patients who suffer from ailments of the kidney.

Unfortunately, this is not the case with streptomycin which does produce kidney irritation. Often patients treated with

streptomycin have albumin and even blood in the urine as the result of kidney irritation. These manifestations are not dangerous, unless the patient had kidney trouble before treatment.

On this question no information is yet available on the newly discovered antibiotics (aureomycin, bacitracin, and others)

Skin rashes are often observed in the course of sulfonamide therapy. They are more severe as a rule, when the treatment is given a second or third time. Some physicians have observed that sun's rays increase the skin rash caused by sulfa drug therapy. The drug which seems to cause skin reaction most frequently is sulfathiazole.

Penicillin also causes skin irritations of various forms, and varying degrees of severity. Between 1 and 2 per cent of men are allergic to penicillin. Among this group are observed the most unpleasant skin rashes and eruptions.¹¹ Streptomycin is also responsible for this type of reaction. Skin rashes of varying degrees of gravity have been described in patients receiving this drug. Sometimes the itching is unbearable, but is rarely dangerous and should not serve to stop the treatment.

General reactions to sulfa drugs vary in frequency and seriousness, depending upon the compound. Vomiting is quite common after taking sulfapyridine, but occurs less frequently with sulfadiazine. Dizziness is a common manifestation with sulfanilamide and sulfapyridine therapy. It occurs much more rarely with sulfadiazine. Drug fever is a usual manifestation in patients receiving sulfathiazole, while here sulfadiazine offends less.

It is estimated that serious toxic manifestations (damage to the blood forming system, or to the kidneys, or to the liver, or other reactions) take place in about 20 per cent of patients submitted to sulfathiazole therapy, in about 17 per cent of cases in sulfapyridine and in 13.5 per cent in sulfanilamide.

treatment. The least toxic compound is believed to be sulfa diazine from which only one patient out of fifteen may experience toxic reactions

Generally reactions in penicillin therapy are serious only when there is an allergy to the drug. There may be a serum like fever with vomiting headache and skin rash. Some mild skin irritation headache and general malaise may occur when penicillin is administered in very large doses.

When streptomycin was introduced into medical practice many cases of histamine like reaction were reported. Heart palpitation rapid pulse and even trembling were the symptoms. However with further purification of the drug this type of reaction has become less frequent. Nevertheless even at present about 7 per cent of patients experience more or less strong toxic reactions. The larger the total dose given the more pronounced the symptoms may be. As with the sulfonamides the length of time of the therapy is more important than the dose of the drug given at one time. Most of the symptoms appearing during and after streptomycin therapy are due to the toxic effect of the drug upon the eighth cranial nerve.

One of the gravest complications which may occur from streptomycin therapy is deafness. It is estimated that about 0.8 per cent or one out of one hundred twenty five may temporarily become deaf if treated with this drug. If the person already has poor hearing streptomycin should be administered with considerable care and not in very large doses. Vertigo is the commonest reaction in streptomycin therapy. About 95 per cent of persons given large doses of the drug for a long period eventually suffer from vertigo. In some patients the symptoms appear very soon after a few days treatment, while others begin to experience toxic effects only after two or three weeks of continuous therapy. These symp



of Tak D & Co

DR H M CROOKS JR
(left) WHO COORDINATED
CHEMICAL TEAM THAT
FIRST SYNTHESIZED CHLORO-
MYCETIN SHOWN WITH DR
E H PAYNE FIRST PHYSI-
CIAN IN HISTORY TO USE
IT ON HUMAN BEINGS



DR WILLIAM REIDSTOCK
FIRST TO SYNTHESIZE
ACTIVE CHLOROMYCETIN



Both ph os f n l k f & C

DIN EHRICH WITH CULTURE MEDIA USED TO GROW FIRST CHLOROMYCETIN

PHOTOMICROGRAPH OF CHLOROMYCETIN CRYSTALS



toms, unpleasant as they may be, are not dangerous and the treatment may continue

Aureomycin often produces nausea and sometimes dizziness of a mild nature Terramycin often causes diarrhea

RESISTANT GERMS

Bacteria submitted to the action of antibiotic substances often acquire a resistance to them Some germs are more apt than others to develop such a resistance which, consequently, makes the treatment, if not a complete failure, at least less effective Germs often develop resistance to some sulfa drugs and particularly to streptomycin

In the case of penicillin such an acquired resistance is less manifest than with the sulfa drugs On the other hand, streptomycin seems to be the substance which induces quite often, and more rapidly a resistance to the germs against which it is given ¹² But an acquired resistance to the antibiotic is not the only problem with which the physician is confronted There are strains among the bacteria which are naturally resistant to this or that drug

The resistant bacteria is the bane of the physician When he prescribes sulfa drugs or penicillin he can never be sure that the infection is not caused by a resistant strain of the germ. Fortunately, however, when a bacteria, for example, the gonococcus which causes gonorrhea, acquires a resistance towards sulfa drugs this does not mean it also becomes resistant to penicillin Quite the contrary, the gonococcus which resists sulfa drugs is as susceptible to penicillin as are non resistant gonococci Some gonococci may become resistant to penicillin if the initial dose of the drug is too small, but since knowledge of dosages is well advanced, such cases are rare

There is always the hope that if one drug fails another may succeed Often, to assure success, a combined therapy is instituted from the beginning of the treatment. However, such

a combined therapy is hardly advisable unless the condition of the patient is grave. In most cases one drug is applied at the beginning of the treatment and if it does not produce the desired effect then it is replaced with another antibiotic.

COMPARISON OF ANTIMICROBIAL ACTIVITIES OF SULFONAMIDES AND OTHER ANTIBIOTICS

By a strange biological paradox, sulfonamides are active against the same groups of bacteria as penicillin. These two antibiotics are specifically effective against Gram-positive germs: staphylococci, pneumococci, streptococci, and gonococci. However, there is little doubt that penicillin is stronger against these bacteria than sulfonamides. Both of these drugs are ineffective against the Gram-negative group of bacteria, with a few exceptions. Sulfonamides are helpful against the germ of dysenteriae, *Shigella dysenteriae*, while penicillin is not.

Penicillin will work against some spirochetes (*Borrelia novyi* and others) while the sulfonamides will not. Penicillin shows some activity against parrot-fever virus, while the sulfa drugs are completely ineffective. The only exception is the recently developed compound phenolsulfazole, which, it is claimed, has antiviral activity.

Streptomycin is active against both Gram-positive and Gram-negative bacteria. As a rule this drug is much less effective against the Gram-positive organisms than penicillin (with the exception of tubercle bacilli). Although streptomycin is active against *Escherichia coli*, the drug is not used for this condition due to its toxicity. It is very effective against *Bacillus proteus* and *Pseudomonas aeruginosa* and is used in urinary tract infections due to these microorganisms. Streptomycin is active against tubercle bacilli and *Bacteria tularensis* (the agent of tularemia), against which penicillin is inactive, or very slightly active.

Chloromycetin, aureomycin, terramycin and polymyxin show activity against many Gram negative organisms, and the first three seem to be effective in some virus infections although their value against influenza A and B and common cold agents is far from established

INFECTIOUS DISEASES

The infectious diseases which affect man are as numerous as the disease-producing germs. For example, infection of the lungs, pneumonia, may be caused by various types of bacteria. Accordingly, the course of the disease and the treatment depend on the bacterium which is responsible. In this book it is not our intention to give a description either of the specific therapies recommended in this or that infection, or the proper method and dosage recommended for any particular disease. We shall limit ourselves to a brief review of what antibiotic seems to be the most effective therapeutic agent in the most common infectious diseases.

Endocarditis may appear in acute or subacute form. The germ, after entering the blood stream, attacks the valves of the heart. There the germs grow, forming vegetations which consist of masses of bacteria encamped in the inflamed tissues of the heart valves. In acute endocarditis there is a real danger that the valves may be destroyed by the infection. Acute endocarditis may be caused by various bacteria, such as streptococcus, gonococcus, staphylococcus, and even pneumococcus. Against most of these bacteria penicillin is effective, but a combined therapy of penicillin with aureomycin, terramycin, or streptomycin is recommended. Subacute endocarditis is also serious and requires long and patient treatment. The offender is, as a rule, *Streptococcus viridans*, or some other streptococci. Penicillin in very large doses, given for a long period of time, brings recovery in about 70 per cent or more.

cases, depending on the type of germ.¹² A combined penicillin and heparin therapy seems to bring better results according to Loewe and his associates.

Meningitis, or the infection of the meninges of the brain was usually a fatal disease before the antibacterial drugs became available. Various bacteria may cause it but the most common offender is meningococcus. This germ is highly susceptible to many sulfa compounds and sulfadiazine often gives good results. But since this bacterium is also highly susceptible to penicillin a combined therapy is as a rule often indicated. In more serious cases penicillin is given by intramuscular as well as intrathecal injections. If the disease is treated promptly there are ninety five out of a hundred chances of recovery.

Meningitis caused by staphylococci is much more dangerous. The offender is usually *Staphylococcus aureus* its origin perhaps a sinus infection, a wound of the skull or mastoiditis. The germ is more susceptible to penicillin than to sulfa compounds. Some doctors have reported 100 per cent recovery with penicillin therapy. In some cases of resistant staphylococcus aureomycin or streptomycin combined with penicillin is being used.

Pneumococcal meningitis is not an infrequent disease. This infection may resist treatment with sulfa compounds for this reason a combined therapy of sulfadiazine and penicillin or aureomycin is generally applied.

Pneumonia or infection of the lungs may be caused by various bacteria but the most frequent offender is *Diplococcus pneumoniae*. This germ is highly susceptible to penicillin. Therefore a prompt recovery is usually effected through penicillin therapy or with a combined therapy of penicillin aureomycin or terramycin.

Pneumonia caused by staphylococci or streptococci is relatively rare but much more dangerous. In staphylococcal

pneumonia penicillin may bring recovery in about 85 to 87 per cent of cases. In streptococcal pneumonia the chances are smaller, about 60 to 65 per cent.

Atypical pneumonia, some types of virus pneumonia, and influenzal pneumonia respond favorably to aureomycin and terramycin as well as to chloromycetin.

Nephritis, or infection of the kidney, may exist either in an acute or chronic form. Acute nephritis (glomerulonephritis) sometimes occurs after a streptococcus infection of the lungs or bronchia. Since penicillin does not damage the kidney and does not produce an irritation of the kidney tubes, it is preferable to the sulfa compounds. In chronic forms of nephritis, however, penicillin is of little help.

Cystitis and other infections of the urinary tract are, as a rule, caused by Gram negative bacteria. Neither penicillin nor sulfa drugs are of much help. Streptomycin is the preferred drug and has proven highly effective in many infections of the urinary tract when the offenders are *Escherichia coli* and *Klebsiella pneumoniae* or *Pseudomonas aeruginosa*.

Syphilis is caused by the *Treponema pallidum*, a spirochete which is susceptible to penicillin. The drug has proven of considerable value in the treatment of fresh infections and has given relief in some forms of chronic syphilis. Against this disease neither the sulfa drugs nor streptomycin, nor aureomycin, nor related drugs are effective.

Gonorrhea the agent of this infection is *Neisseria gonorrhea* which is highly susceptible to penicillin. Penicillin is the preferred remedy, although sulfadiazine shows considerable activity against this infection.

Bronchitis, or infection of the bronches, may be caused by various microorganisms. Among the most frequent offenders are streptococci (of the hemolytic type) and *Staphylococcus aureus*. Penicillin alone, or in combination with other drugs, such as aureomycin, may bring some relief.

Dysentery, an intestinal infection, is caused by a genus of bacteria called *Shigella* (*Shigella shigae*, *Shigella flexneri*, etc.). Penicillin and streptomycin are ineffective, while the sulfa compounds, particularly sulfamerazine, seem to be of considerable value.

Influenza and the *common cold* are caused by virus organisms. Aureomycin, terramycin, and chloromycetin seem to give considerable relief in these conditions without being completely effective.

Brucellosis, otherwise known as undulant fever, is caused by *Brucella abortus* or *Brucella suis*. Streptomycin combined with sulfadiazine has given very encouraging results, although toxic reactions of considerable gravity have been reported.

Tularemia is caused by the microorganism called *Bacterium tularense*, which is transmitted to man from wild rabbits. Streptomycin is effective but aureomycin even more so. Penicillin and sulfa drugs are not.

Sore throat, this common affliction, is caused usually by streptococcus. It is often blamed for the many grave complications due to this germ. Penicillin is the most effective remedy if given by injection and simultaneous local application.

In many other infectious diseases not mentioned in this brief review penicillin, the sulfonamides and streptomycin may be useful and even highly effective if the causative agent is susceptible to the drug. In carbuncle, various abscesses, osteomyelitis, otitis, and some skin infections one or more of these substances have proven of high efficiency. Penicillin and the sulfonamides are being used with a high degree of success in infections of the mouth, throat, ear, eye and larynx, as well as in vaginal infections. In many other diseases, such as typhoid fever, typhus fever, colitis, rheumatic fever, and cholera none of these drugs have proven effective. On the other hand, some of the newly discovered antibiotics are quite promising in this respect. Aureomycin seems to possess some

antivirus activity, while chloromycetin and polymyxin show considerable activity against some of the Gram negative bacteria

Antibiotics are playing an increasingly important role in preventive medicine. Not only in post-operative treatment, but in every day medical practice they have become an essential weapon in reducing infectious diseases and their complications. As an example we may give the treatment of sore throat with penicillin. It arrests the infection over night and often prevents dangerous complications of this affliction, in itself innocuous. Even more impressive is the fact that penicillin is of value in preventing recurrent attacks of rheumatic fever. Moreover, rheumatic fever itself can be prevented if sore throat caused by streptococcus is promptly treated with penicillin. More and more antibiotics are being used for the prevention of disease. Consequently, the mortality rate for scarlet fever, whooping cough, pneumonia, and peritonitis were at an all time low during 1949 (Bull. Metrop. Life Insur. Co. 30 3, 1949).

Summing up the achievements in the chemotherapeutic field during the last ten years many infectious diseases, which a decade or more ago were considered incurable, and which have been responsible for high mortalities, are now virtually conquered.

While there remain a number of infectious diseases which cannot be vanquished by the drugs so far known, we are still only at the beginning of the new era of chemotherapy, each year, if not each month, comes encouraging news about some newly discovered antibiotic. With the investigations going forward so intensively and so rationally, in the field of chemotherapy, one cannot doubt that sooner or later our armament of antimicrobial substances will become so rich and powerful that only a few, if any, disease producing germs may remain beyond the reach of the weapons in the arsenal of science.

16

Man Against Microbe

MEDICAL RESEARCH has the greatest fascination for men of science. Every discovery in the medical field brings us closer to a better and more profound understanding of the human body on the one hand its weaknesses and limitations on the other its powers of resistance and amazing ability to recuperate.

Medical scientists gain satisfaction often denied to their colleagues in other fields from the knowledge that they are helping to alleviate suffering and to cure illness. In ascertaining the origins of disease in discovering new methods of fighting afflictions in reducing the death rate and in prolonging man's lifespan medical scientists have already accomplished a near miracle in the last half-century. The progress though astonishing is still going forward with gigantic strides checked neither by wars nor by other catastrophes of mankind's turbulent existence.

Decades ago infectious diseases were accepted fatalistically as the will of heaven. Man was powerless against them. Epidemics of scarlet fever cholera or typhus preyed upon mankind pitilessly and almost without opposition. But now most

of these dangerous and swiftly progressing infectious diseases are under control

In the maternity wards of Europe the mortality rate among newborn infants was once as high as 50 per cent. At present only a few among thousands die in early infancy.

During World War I wound infections in the fighting forces caused vast numbers of deaths. In some armies almost 70 per cent of the wounded died because of acute infections of wounds and fractures. In this respect, World War II casualties were reduced impressively. Not only in our own and in the British armed forces, but in the Russian armies as well, the number of men who died from wound complications was sharply diminished.

For this, and many other achievements in the field of practical medicine and surgery, thanks are due to a small army of medical scientists laboring in the laboratories and scientific institutions of the world. Their names, with few exceptions, remain unknown, not only to the general public but even to the medical profession, which so freely makes use of the fruits of their investigations. As a rule these scientists are poorly paid, their incomes being but a fraction of the earnings of their colleagues who are practitioners. Nevertheless, they have more contentment and satisfaction than the workers in almost any other field. Their tasks are always intriguing and exciting, for they deal with the most fundamental problems of existence, the eternal questions of life and death.

In every medical discovery there is the 'continuation of the idea.' The discovery is never regarded as completely finished or accomplished. It is endlessly redeveloped, with ever new lines of research branching out. Each discovery leads to a new investigation, and each investigation to a new discovery. This never ending, engrossing, and exciting penetration into the mysteries of life stimulates man's curiosity and imagination and beckons him further.

Medical research is like a growing plant. From a single seed sown by an unknown, perhaps long-forgotten scientist, gradually burgeons a gigantic tree, with numerous branches and limbs. Its appearance in the world is slow, and often has to overcome many obstacles. The ground for a new discovery must be well prepared. Before the discovery matures, the idea itself must germinate a long time. In this preparatory work, any number of scientists may take part. Here and there they toil independently of one another, until the moment arrives when a man of dynamic personality and vision unites and synthesizes the isolated findings, and brings the discovery to the public.

Laymen hear only the names of the great luminaries, the scientists who promote the success of a discovery and bring it to its happy conclusion. The public is familiar with such names as William Harvey, Joseph Lister, Louis Pasteur, Robert Koch, Paul Ehrlich, Theobald Smith, or Frederick Banting. But behind these were thousands of other scientists who not only prepared the ground, but in some cases actually did the initial and embryonic work long before it was at last disclosed and accepted.

In medical research there is no such thing as a wholly unexpected discovery. Almost every important disclosure is foreseen and predicted. The germination of an idea can often be traced to an obscure or forgotten source.

Who has not heard of the work of Dr. William Harvey, the father of physiology? In the seventeenth century, he discovered and properly described the circulation of the blood and thus laid the cornerstone of modern medical science. Yet the medical men of the University of Padua, where Harvey had studied medicine as a young man, were very close to the discovery of the same principles. Had not Servetus already written about the circulation through the lungs? Had not Fabricius, Harvey's instructor, discovered and described the

valves of the veins? The ground for the new theory of circulation had been prepared. But it was Harvey who coordinated the existing evidence and made of it a whole, who found new proofs and, by simple and ingenious experimentation, laid the foundation for his new theory.

Bacteria were discovered by Anton von Leeuwenhoek in the year 1676. But years before, Pierre Borel, using an elementary microscope, observed bacteria like organisms which he described in his book *Historiarum et Observationum Medicophysicarum*, published in 1653. In 1650 Athanasius Kircher claimed that he saw in blood the microscopic organisms which were, in his opinion, the living agents of the plague.

And Pasteur himself? Long before he was able to prove that germs were the cause of infection, he was convinced that the idea of spontaneous generation of bacteria was wholly erroneous. There was already ample evidence to suggest that bacteria, from the air or through direct contact, infected wounds, penetrated the blood stream, and induced all sorts of infectious diseases. Twenty years earlier Agostino Bassi had described the microorganism *Botrytis bassiana*, which caused the disease of silkworms. At the same time E. Eichstadt discovered a germ of contagious skin disease known as pityriasis.

Fully forty years before Pasteur began his experiments on anthrax N. Barthelemy demonstrated that the germ of an thrax could be transmitted to sheep. After him, A. Rayer and C. J. Davaine were able to prove that this disease could be transmitted from infected to healthy sheep by blood inoculation. Fifteen years later Pasteur isolated the germ, cultivated it, and proved that the infection was caused by bacteria. Pasteur's greatness lay not only in the observations he made, and the experiments he undertook, but in his courage, the brilliance of his inductive reasoning, his vision, and his fighting spirit. He would fight for an idea in which he believed.

until he could bring it from obscurity to general acceptance.

Even the momentous discovery by Alexander Fleming of penicillin, the drug which has brought us so much closer to a successful mastery over the world of microbes had its fore-runners. As early as 1897 C. Duchesne reported that a certain mold belonging to *Penicillium* was capable of inhibiting the growth of germs. Fifteen years later, A. Vaudremer, of the Pasteur Institute, made the discovery that a mold, *Aspergillus fumigatus*, exercised a depressive action on tubercle bacilli, rendering them less virulent and aggressive. A few years before the now famous mold *Penicillium notatum* was discovered, L. Rosenthal at the Pasteur Institute in Paris and Andrea Gratia and Sara Dath at the Pasteur Institute in Brussels actually discovered and described the antibiotic substances produced by mold bacteria. But all the discoveries which preceded Fleming's work only indicated that the idea was in the air, awaiting some great scientist who would crystallize it. The greatness of Fleming lay in his unlimited belief in the rightness of his discovery, in his vision and in his stubborn persistence.

Thus there is some element of clairvoyance in the progress of medical science. There is a goal toward which medical bacteriology guides us. What is this goal of the science which studies disease-producing germs in order to fight them more efficiently?

There was a time when prehistoric man lived in a wilderness of forests surrounded by dangerous beasts. He considered his battle for existence normal and natural. One day the beasts would kill and devour a man; the next day a man would kill and devour a beast. Primitive man was never able to visualize any other, less dangerous existence. With the development of civilization and concentrations of population the danger from wild animals has disappeared. Some animals have become domesticated and serve the needs of man. But

as the result of civilization new menaces appeared in the form of disease producing germs. Infectious diseases almost unknown to our ancient ancestors—at least as far as our records show—began to endanger the existence of the human race. The most ferocious epidemics devastated town and country. Through the ages, epidemics of plague, cholera, typhus, diphtheria, and smallpox took a toll of countless millions. Pneumonia and other infectious diseases were incurable and men died young or in the prime of their lives.

What was the attitude of man toward these new perils, the nature of which he barely grasped? Danger from the wild beasts was a physical reality which man could face and combat, but infectious diseases confronted him with *the unknown*. Man's complete helplessness against the sudden epidemics which killed wives, husbands, children, and friends made these epidemics seem supernatural, as though sent by Providence. Throughout the centuries, the theurgical theories of the origin of contagious disease in general, and of epidemics in particular, dominated his thinking. Microbes, invisible, omnipotent foes, more dangerous than any wild beasts, were everywhere around him. Disease was looked upon as the striking arm of Providence, the punishment for sin and immorality. Even when, with the progress of science, we acquired full knowledge of the disease producing germs, the fatalistic attitude toward infectious disease still remained. Although modified in some degree, this belief was still part of our consciousness until the time of the discovery of penicillin.

The extraordinary role which antibiotics, particularly penicillin, have played, has not yet been fully evaluated. The *brief years which have elapsed since penicillin was first tested* have radically changed our attitude toward our old enemy, the microbe.

The psychological factors in the fight against disease are

of prime importance. The disappearance of our fatalistic attitude toward infectious diseases as the result of the discovery of antibiotics, and our conviction that the microbes can be fought successfully with these miracle drugs greatly contribute to prompt recovery. Of course, the defense forces of the organism are still the major resources in the fight against infection, but the improvement in the morale of the patient does often make the difference between success and failure in the treatment.

What is the goal toward which medical bacteriology is heading? It sounds like a dream or a fairy tale: this goal towards which medical science is moving as though propelled by Destiny. The goal is simply *to live in a world without menacing microbes*, to have all disease-producing microbes rendered harmless and domesticated, to see infectious diseases vanish from the earth or at least be easily controlled to make this planet free from the dangers of death from infectious diseases so that the common cold, pneumonia, plague, meningitis, tuberculosis and other dread ailments may be as rare phenomena as dangerous wild beasts are.

Will such a world ever exist? We believe so. All the evidence supports the contention that sooner or later we will no longer need to fear disease-producing germs. True, we stand only on the threshold of this new era and perhaps many decades may pass before the victory is within our grasp. But there is no doubt, no hesitation in the mind of anyone who watches closely the progress of the new science of antibiotics that this dream will yet be realized by men of science.

During the recent past a gigantic step has been made in this direction. We have learned much about the intimate mechanism of the life of pathogenic bacteria, their weaknesses and limitations in regard to the antibiotic substances. By the discovery of penicillin and other substances produced by molds and bacteria a new road has been opened. In this re-

spect more has been accomplished for chemotherapy during the past few years than for decades previously. Alexander Fleming has earned the tribute which Lister paid Pasteur: 'You have raised the veil that for years has covered our fight with infectious disease.' For Fleming has pointed out the trail for new and more effective methods of hunting microbes, the path which cannot help but lead to ultimate victory.

Moreover, the new trend in chemotherapy is of far reaching significance for human beings in other ways. The role of microbial activity in the human body is not limited to acute infectious diseases. Sooner or later the antibiotics will find their application in the treatment of many chronic diseases such as rheumatoid arthritis. For the source of this disease is an infectious process of a latent type. A slight cold, or sinusitis or, in fact, any infection may have a damaging effect upon the vital organs of the body. Disturbances of the glandular system, particularly those of the thyroid and adrenal glands, frequently occur as a result of infection. Also, in the numerous cases where a prompt liquidation of the infection may prevent a chronic ailment, the role of antibiotics will be of great service to the human race, and will gradually contribute to the improvement of general health.

In the light of the discoveries of antibiotics the future of mankind appears quite hopeful. Each year the mortality from these diseases will decline, and the menace from the pathogenic microbe will diminish. Small wonder that man has hailed these discoveries as 'miracle drugs'.

Footnotes

CHAPTER 3

- 1 Twort F W, *Lancet*, 2 1241 1915 2 1064 1930
- 2 d'Herelle, F, *Compt rend, soc biol*, 165 373, 1917, see also "The Bacteriophage and Its Behavior" Baltimore 1926
- 3 d'Herelle F., *Internat Clinics* 1 36 1924
- 4 Bordet J, *Ann de l'Institut de Pasteur* 42 1283 1928
- 5 *Deut med Wochschr*, 61 250 253 1935, also in *Angew Chem*, 48 657 1935
- 6 *Compt rend soc biol* 120 756 1935
- 7 *Min Wochschr*, 16 1412 1937
- 8 They prepared two substances 2 sulfanilamidopyrimidine and 2 sulfanil amido-4 methylpyrimidine See Chemotherapy 11 Some Sulfanilamido Heterocycles *J Amer Chem Soc*, 62 200* 1940
- 9 *Bull Acad Med*, 8 260 18,9.
- 10 Ogston A *British Med J* 1 369 1881
- 11 They are inactive in vivo against all spirochetes all viruses and all G am negative bacteria with few exceptions
- 12 Fischer K S and Armstrong F *J Gen Physiology*, 30 263, 1917
- 13 Mlotz I M "The Mode of Action of Sulfonamides" *J Am Chem Soc*, March, 1944, p 459 He concluded that the inhibition of bacterial growth by sulfonamides may be accounted for quantitatively by assuming that the action is due to a reversible combination between the basic form of the drug and the neutral form of the protein
- 14 *J Exper Med*, 79 331, 1944
- 15 Housewright R. D and Koser S A., *J Infect Dis*, 75 113 1944
- 16 Zeller, W W *et al*, *JAMA* 136 12 1948
- 17 Medin O *Verhandl d.X internat med Kong* 2 37 1890
- 18 Quoted from *Science News Letters* July 31, 1948 and September 11, 1948
- 19 Gino Brogi *Pediatrics* 49 573 1941
- 20 They reported on the occurrence of diffuse necrotic and degenerative lesions in patients who received a sulfonamide drug prior to death *Am J Path*, 22 66, 1946

- 21 They reported granulomatous and polyvascular lesions in the patients treated with sulfonamides. *Am J Path.*, 22 679 1916 also. 22 703 1916

CHAPTER 4

- 1 From Metchnikoff's conversation
- 2 From conversation with Dr. Beredka.
3. Dry substance constitutes 14 per cent of the tubercle bacilli one-fourth of which is soluble in alcohol and ether consisting of free fatty acids and fatty acids combined with the higher alcohol "mykol" to form a wax
- 4 The tubercle bacilli are not always destroyed by the gastric juice in the stomach as is shown by successful infection experiments in susceptible animals fed with tubercle bacilli William H Park Anne W Williams, and Charles Kumwiede, *Pathogenic Microorganisms* (Philadelphia Lea and Febiger 1921)
5. Serge Metchnikoff, *L'infection Microbienne et l'Immunité* (Paris: Masson and Company 1927)
- 6 There was present a lysis of the tubercle bacilli in the abdomen of *Galleria mellonella*
- 7 While Metchnikoff was trying to solve the problem of the treatment of tuberculosis by dissolving the bacillus capsules A. L. Vaidremer also working at the Pasteur Institute made an interesting observation. He discovered that tuberculin, when added to the filtered extract of *Penicillium glaucum*, loses its activity. Thus it was thought that there might be something in the mold which is antagonistic to the tubercle bacillus. This observation made in 1910 attracted little if any attention. The idea seemed to be too absurd.
- 8 *L. acidophilus* and the closely related type, *L. bifidus* which occur abundantly in the intestines of breast fed infants are typical intestinal organisms. Genuine *L. acidophilus* of human origin differs from *L. bulgaricus*, which is rapidly destroyed in the intestines. This fact was of course not known to Metchnikoff who advocated the implantation of *L. bulgaricus*. Successful implantation of *L. acidophilus* depends largely on the strains of the organism which are selected for the purpose.
9. L. F. Rettger and H. A. Cheplin *The transformation of intestinal flora, with special reference to the implantation of Bacillus acidophilus* (New Haven: Yale University Press 1921) Also L. F. Rettger M. N. Levy L. Weinstein and J. E. Weiss *Lactobacillus acidophilus and its therapeutic application* (New Haven: Yale University 1935)
10. Emmerich Löw and Korschun discovered and described the peculiar properties of *B. pyocyaneus* in 1899
- 11 Pasteur first applied the principle of protective inoculation to the prevention of anthrax. He was able to make animals immune by a series of inoculations with anthrax germs whose disease-producing power had been so reduced that the injections caused no harm. The preparation of anthrax germs used for these inoculations was called anthrax vaccine.
- 12 Schoental who is an associate of Dr. Howard Florey recently has reviewed the work on *B. pyocyaneus* which is one of the oldest known examples of a microorganism producing substances antagonistic to other bacteria.

- There are three different substances produced by this bacterium pyocyanin alpha hydro phenazine and an almost colorless compound all of them being isolated from a chloroform extract of *B. pyocyaneus*
- 13 According to these authors the *B. pyocyaneus* is able to produce its blue pigment in the absence of all nutritive material except the ammonium salts of the dibasic acids of the succinic series
 - 14 According to Soresina pyocyanin stimulates cell respiration and inhibits glycolysis In vitro it increases cell multiplication
 - 15 See the work of Carlson and Bodine F Dickens C. W. Wagner We should also mention the attempt to treat cancer with the substances produced by some bacteria About fifty years ago Dr William B. Coley of New York developed a method of treatment in which he employed a combination of toxins composed of sterile filtrates of the streptococcus of erysipelas and *Bacillus prodigiosus* He claimed encouraging results in about 20 per cent of the cases treated by this method. William L. Laurence recently suggested that cancer cells require biotin (a vitamin of the B complex) and that the erysipelas streptococcus has "an avidin like action" that deprives the cancer of this vital substance It was therefore suggested that large amounts of raw egg white would produce the same results as erysipelas. This hypothesis was tested at Bellevue Hospital New York by Dr Ira I. Kaplan with encouraging results reported by him in the June issue (1945) of the *American Medical Journal of the Medical Sciences* These results which may be due either to the action of the free avidin or to the lytic action on cancer cells of lysozyme or to both lend some support to the suggestion that hyaluronidase which acts in a manner similar to lysozyme is the enzyme in the erysipelas streptococcus responsible for its anticancer action Other investigators however have not confirmed Dr Kaplan's results
 - 16 L. Rosenthal investigated the antibacterial activity of *Tyrophthrix tenuis* *scaber* *filiformis* *geneculatus* *distortus* and *minimus*

CHAPTER 5

- 1 Alexander Fleming *Proc Roy Soc Med* 46 71 1932
- 2 Gratia and Dath showed that *Penicillium glaucum* is able to dissolve *Clostridium welchii* the bacterium responsible for gas gangrene. The interesting fact is that we have learned recently that penicillin is effective against the germs of gas gangrene in some instances
- 3 It is established that there is more than one agent which initiates in influenza A virus like organism is now considered the most important cause of influenza epidemics
- 4 Karl Meyer "The relationship of lysozyme to avidin" *Science*, 99 391 1944 Also William L. Laurence "On the possible identity of avidin and egg white lysozyme" *Ibid* These investigations provide evidence for the first time that the active principle in lysozyme is histon that avidin an other mysterious substance isolated from egg white is a component of lysozyme serving as the carrier or binder in an enzyme system that makes histon available to the tissues and organs of the body for proper normal functioning

In one set of experiments a sample of lysozyme prepared by Dr Meyer was tested to determine whether or not it contained avidin the substance that combines in a strong chemical union with biotin. In another series of experiments samples of avidin concentrates were tested for possible lysozymic activity. Both series of tests yielded positive results showing that the lysozyme concentrate contained avidin and that the avidin concentrate possessed strong lysozyme activity by dissolving the bacteria used as test organisms. The avidin activity was found to be proportional to the lysozyme activity.

Since free avidin combines with biotin Dr Meyer added varying amounts of pure biotin to the avidin concentrates to see what the effect of such additions would be on the lysozyme activity of the concentrates. The tests revealed that the addition of ten millionths of a gram of biotin increased the bacteria dissolving power of the lysozyme from 8 to 250 times. Furthermore the power of the lysozyme to dissolve 7.5 per cent of the bacteria was increased more than 500 times.

5. The cup-plate method of assay is generally considered to be the standard method and is the official method used by the Food and Drug Administration for penicillin assay. See the article by W. H. Schmidt and A. J. Moyer *J. Bact.* 47:199-210, 1944.
6. Dr Florey demonstrated that pure penicillin produced morphological effects on streptococcus in dilutions of 1 to 250 million. *J. A. M. A.* 124:117, 1934.
7. L. A. Rantz and W. M. Kirby have found that penicillin is actively bactericidal for the staphylococcus and that lysis of the organism occurs as a result of penicillin action. In spite of the ease with which enormous numbers of staphylococci can be killed by small amounts of penicillin many organisms remain alive even after prolonged exposure to this chemical. On retesting the remaining viable bacteria may be shown to be as sensitive to the action of penicillin as was the parent strain so that their survival is not the result of artificially induced penicillin resistance. The presence in the blood and tissues of 0.1 and 0.2 unit of penicillin per cubic centimeter would seem to be adequate for the therapy of most clinical infections. It is generally stated that penicillin is not inhibited by serum, body fluids or peptones. However lysis occurs somewhat more slowly and more organisms remain viable after prolonged exposure to penicillin if a rice broth is used rather than the relatively incomplete synthetic medium.

When peptones were added to the synthetic medium in increasing concentration the control organism multiplied more rapidly but penicillin activity was unimpaired. This is in striking contrast to the action of the sulfonamides. It is possible that the constituents of the culture medium have no effect on the inhibitory phase of penicillin action on the staphylococcus but are concerned in the ease with which the agent may induce lysis and killing of the bacteria. Penicillin is an extraordinarily potent agent, which in minute amounts induces the death and lysis of staphylococci. That this effect is not complete and that viable organisms remain after prolonged exposure to the drug is unfortunate and may explain certain clinical failures the clinical significance of which has not

been evaluated H T Helmholtz and Chieh Sung have demonstrated a weak bactericidal effect of penicillin in urine on *Streptococcus fecalis* and on *Proteus ammoniae*. The work of G L Hobby indicates that penicillin possesses an antibacterial action in vitro against some Gram negative organisms "It is possible, according to this author, "that a form of penicillin showing greater activity against Gram negative organisms may exist."

- 8 Penicillin at a dilution of 1:100 killed leucocytes immediately at 1:250 more than 50 per cent lived for four hours. The preparation at 1:500 is harmless.
- 9 The experiments strongly indicated that there was no antagonistic effect when penicillin was admixed to the blood. See also the work of Rammelkamp on this subject.
- 10 It appears that penicillin is even less toxic to macrophages than to fibroblasts. Dr Ivor Cornman stated that in roller tube cultures untreated sarcoma cells grown with normal fibroblasts derived from the same strains of tumor host grew fully as vigorously as the normal cells. However upon addition of penicillin the sarcoma cells were selectively damaged. With proper choice of the dosage level it was found possible to kill all the sarcoma cells without damaging the normal fibroblasts. But according to Margaret Reed Lewis penicillin by itself does not exert any retarding effect upon the growth of graft of sarcoma in mice. It seems that some unknown substance which is present in penicillin compounds might be responsible for this action.
- 11 See the work of Rammelkamp and Keefer. Traces of penicillin were found in the blood as long as 210 minutes after intravenous injection. Penicillin does not penetrate the red blood cells in significant amount. The concentration in the red cells is about 10 per cent of that in the plasma.
- 12 M J Romansky and G E Rittman have disclosed that penicillin in beeswax peanut oil mixture exercises much more prolonged effect than regular penicillin solution. M Tromper and A Hutter suggest a very ingenious technique to prolong the effectiveness of penicillin action with ice application in the site of the injection of penicillin. They call it the chilling technique.
- 13 The investigation of A H Free, J R. Leonards, D R. McCulloch and B E Bio conducted on normal human subjects indicates that not all penicillin is destroyed by gastric juice. According to these authors from 8 to 33 per cent of the quantity of penicillin taken by mouth was excreted in the urine. The maximum excretion occurred during the first hour and all penicillin disappeared from the urine by the end of six hours. An attempt to give penicillin together with bicarbonate of soda was a complete failure. All penicillin seemed to be destroyed in the intestines.
- 14 The base for this paste was lanette wax.

CHAPTER 6

- 1 It must be said that while only two strains of *Penicillium notatum* are used for the production of penicillin many other penicillia are capable of producing this substance. Having examined about 240 different cultures of

- this group of mold Raper, Alexander, and Coghill have found that about 90 per cent of them produced some amount of penicillin
- 2 "The operation of large vat fermenters under absolutely aseptic conditions is beset with many difficulties and the history of the development of the submerged production of penicillin has been a succession of heartaches and disappointments for those concerned" (R. Coghill)
 - 3 Foster, Woodruff and McDaniel advised the use of 1 to 3 mg of zinc sulphate per litre See *J. Fact* 46 481 1943
 - 4 Kocholaty suggested substituting manganese for iron See *Science*, 97 186, 1943
 - 5 See the work by Florey *et al.* Further Observations on Penicillin etc.
 - 6 Meads *et al.* stated that penicillin λ is not absorbed as well and as completely as penicillin G See *Science* 103 301 1943
 - 7 Kirby gives a complete report on this subject. See *J.A.M.A.*, 125 618 1944.
 - 8 Meyer and Hobby point out an interesting fact that the number of surviving bacteria decreases by geometric units as times increases by arithmetic units. This means that more and more germs die faster as time goes on. The log of the number of survivors plotted against time follows a straight line until at least 99 per cent of the microorganisms are destroyed.
 9. A 2 per cent solution of this compound can be prepared in sesame oil. Pure benzyl-ester penicillin was found of much lower activity than the sodium salt of penicillin but when dissolved in sesame oil it seems to increase its activity
 - 10 It was reported that in vitro penicillin is highly bacteriostatic and bactericidal against *Brucella abortus* and *Escherichia coli*. However this compound shows much less activity in vivo against these microorganisms
 - 11 See the reports by Bondi and Dietz who have found that some strains of staphylococci highly resistant to penicillin produced penicillase. *Proc Soc Exp Biol & Med* 56 135 1944. We may note that *Paracolon bacilli*, *Shig dysenteriae* and *Shig paradyenteriae* produce penicillase.
 12. Knox also stressed the fact that young bacteria are particularly susceptible to penicillin and may easily perish while old bacteria show much more resistance to this drug See *Lancet* 1 559 1945
 - 13 All evidence points out that at least a part of bacteria submitted to the action of penicillin undergoes lysis

CHAPTER 7

- 1 Rantz and Kirby have observed even higher figures for the resistant strains of *Staphylococcus aureus*. According to these authors 24 per cent of 4 in reisolated strains have proved to be resistant to penicillin. Apparently 12.9 per cent of this bacterium possess natural resistance toward penicillin and about 9.4 acquire such a resistance
- 2 Penicillin is less active against some groups of *Streptococcus pyogenes* (B, C, E, and G). *Streptococcus salivarius* and *Streptococcus fecalis* show considerable resistance toward the action of penicillin. See the work of Dawson, Hobby and Lipman. *Proc Soc Exp Biol & Med.*, 56 101 1944
- 3 See Jawetz. *Arch Int Med.*, 77 1 1946

- 4 Mice infected with sulfonamide-resistant strains of Types I II and III pneumococci responded promptly to penicillin given by mouth
- 5 Mice infected with a very virulent type of meningococci recovered from the infection after treatment with penicillin See Miller and Foster *Proc. Soc. Exp. Biol. & Med.*, 56 166 1944
- 6 Some strains of *C. diphtheriae* are destroyed by a very small amount of penicillin according to the work of Young and Mood *J. Bact.*, 50 205, 1945
- 7 While McKee and his associates found the activity of penicillin very low against *T. pallidum* Engle and Musselman and others reported that the drug is highly spirocheticidal.
- 8 A distinction must be made between bacteriological "cure" and clinical "cure" Thus Ercolli and Lafferty reported that penicillin even in large doses has not achieved the bacteriological "cure" of rabbits.
- 9 *Brucella suis* seems to be the most susceptible to penicillin among this genus.
- 10 Penicillin has some inhibiting action in vitro on some varieties of *R. prowazeki*
- 11 Penicillin is ineffective against the virus of epidemic influenza according to the investigation of Robinson *J. Pharm. & Exp. Therap.* 77 70 1945
- 12 Heilman and Herrel stress the fact that "there is evidence that the ornithosis virus may frequently cause atypical pneumonia in man. The disease may occur in a mild form and last one or two weeks. The infection however at times may be severe and terminate fatally."
- 13 According to Cooke and Goldring subcutaneous injections given to infants and children gave very satisfactory results. See *J.A.M.A.*, 127 80, 1945
- 14 McClosky and Smith have been able to induce a pronounced sensitization in guinea pigs. See also John Strazza *J.A.M.A.*, 150 1071 1946 Kolodny and Danhoff *J.A.M.A.*, 150 1028 1946 and Peck et al *J.A.M.A.* 158 631 1948
- 15 See *J.A.M.A.*, 154 1546 1947
- 16 According to Hobby and Dawson sulfadiazine appeared to decrease the effectiveness of penicillin against *Staphylococcus aureus*
- 17 Schwartzman reported that the addition of methionine threonine and methiosulfoxide has greatly enhanced the activity of penicillin

CHAPTER 8

- 1 *J. Clin. Invest.* 20.434 1941
- 2 *J.A.M.A.*, 122 1217 24 1945
- 3 Sulfonamides are relatively effective in septicemia caused by hemolytic streptococcus but much less effective in staphylococcal blood infections.
- 4 Bloomfield et al. analyzing the causes of the failure of penicillin in some cases in their article "A Study of Penicillin Failures" point out that "the time factor is likely to be more important" than even the dose itself. The adequate dose however is of prime importance as well.
- 5 When the patient is very gravely ill intravenous injections of penicillin should be combined with intramuscular. It is said that in some cases a combined therapy of penicillin and sulfadiazine gives the best results.

6. In a few cases of psittacosis some improvement was observed but not a complete recovery See Turgasen *J.A.M.A.* 126 1350 1944 Ford and Kispert *Wisc Med J.* 44 991, 1945 and Flippin *J.A.M.A.*, 129 280 1945.
- 7 In intrinsic cases of asthma about 70 per cent of recovery was reported See Barach *et al.*, *Ann Int Med.*, 22 485 1945 Vermilye *J.A.M.A.*, 129 250 1945
8. Multiple abscesses of the lungs are often due to a mixed infection. A combined penicillin and sulfadiazine therapy might be more effective than penicillin alone.
- 9 Sulfadiazine therapy by itself gives the same rate of recovery if not better than the penicillin therapy
- 10 A combined sulfadiazine and penicillin therapy might give a higher rate of recovery from pneumococcal meningitis
- 11 The hemolytic strain of *Staphylococcus aureus* is usually the cause of staphylococcal meningitis Sulfonamide therapy gave not very encouraging results. Some of the authors reported recovery in 100 per cent from penicillin therapy while others (Keefer *et al.*) only in 40 per cent
- 12 An infected tooth is commonly the original source of subacute endocarditis.
- 13 Charles K. Friedberg *J.A.M.A.*, 144 527 1950
- 14 These figures were disputed by other investigators who stressed the fact that the dose of penicillin used by Dr Koch *et al.* was not sufficient
15. Mahoney *et al.*, *Ven Dis. Inform.*, 24 355 1943
- 16 E. Peterson *et al.*, *J.A.M.A.* 144 621 1950.
- 17 Strock, A. E., *J Am Dent Assoc.*, 51 1235 1944

CHAPTER 9

- 1 The investigation of Dr Hopkins is considered as not sufficiently convincing because some of the control patients were not clinically typical cases of the common cold See *Lancet* 2 631, 1943
- 2 These workers arrived at the conclusion that there was no actual difference between the course of the common cold in treated and untreated patients. See *Lancet*, 2 634 1943
- 3 Also the investigation of Stansfeld *et al.* See *Lancet* 22 970 1944
- 4 It is effective in vitro against *Trichophyton gypsum* and *Monilia albicans* See Herrick *Proc Soc Exp Biol & Med.*, 59 41 1945
- 5 Chain and Florey See *British J Exp Path.*, 25 202 1944 also *Lancet*, 1 112 1944

CHAPTER 10

- 1 According to Keefer *et al.*, the incidence of toxic reactions have progressively diminished with the perfection of methods of purification. In the treatment of 1,000 cases the incidence occurred in 20.5 per cent when the daily total dose was over 1.0 gram. But among patients receiving 4.0 grams or over, the incidence of toxic reactions was as high as 60 per cent
- 2 The reactions are usually transient in nature although as the *Journal of the American Medical Association* recorded, two deaths have occurred

from histamine-like reactions. (Editorial, *J.A.M.A.*, 130 939 1946) But these incidents might be explained by impurity of the drug used in the first clinical observations

3. H J Corper and Maurice L. Cohn (*J.A.M.A.*, 137 357, 1948) remark that "streptomycin properly administered and in proper dosage is capable of retarding the development or growth of human tubercle bacilli both in vitro and in vivo. But they add "The concentration of streptomycin required to retard the growth of tubercle bacilli in vitro in good nutrient medium can only be attained in vivo for a brief period by any route of administration or amount of streptomycin that can be used even with the present high assay unit preparations. . . There is no evidence available at present to indicate that tubercle bacilli absorb or adsorb streptomycin or that it accumulates in these bacilli in vitro or in vivo" P 358
4. From the discussion, 96th Annual Session of the American Medical Association (*J.A.M.A.*, 137 357, 1948) Dr Bogen enthusiastically endorsed streptomycin. He stated "We have many guinea pigs surviving after treatment with streptomycin whereas the controls similarly inoculated died many months before. Guinea pigs treated with large doses of streptomycin show minimal lesions. In some cases we have not been able to find any lesions where the controls were riddled with the infection. Similarly, many patients now show complete healing of tuberculous lesions which had previously existed, whereas control patients with similar lesions still show persistence of such lesions."
5. See the report of the Committee on Chemotherapeutics and Other Agents of the National Research Council, *J.A.M.A.*, 132 4 1946
6. There is no radical sterilization because in spite of the prolonged survival of the mice, their lungs continue to be virulent to guinea pigs. Discontinuation of the treatment is often followed by grave recurrences. The identity of the conclusions drawn from these experimental results and those reached from clinical observations in men is striking. See *Presse Medicale*, 55 609 1947
7. According to Corper and Cohn "streptomycin apparently did not affect the viability of a virulent human tubercle bacilli in vivo." See *J.A.M.A.*, 137 362 1949
8. The streptomycin used in the experimental and clinical studies at the Mayo Clinic was derived from several sources. The earliest experimental studies were carried out with the one supplied by Dr S. A. Waksman, discoverer of streptomycin.
9. Nevertheless according to these authors "streptomycin is an antibacterial agent which possesses the unique ability to inhibit the growth of *Mycobacterium tuberculosis* in vivo, both experimentally and clinically. The manifestations of tuberculosis may be suppressed both in experimental animals and in man with at least temporary retardation of the pathologic processes as judged by objective criteria."
10. K. H. Pfuetz and M. M. Pyle "Streptomycin in the Treatment of Tuberculosis" *J.A.M.A.*, 139 634 1949
11. E. J. Beattie, Jr., and Brian B. Blades "Use of Streptomycin in Surgical

- Patients," *J.A.M.A.*, 139 902 1949 Streptomycin was also used in the treatment of *lupus vulgaris* See T Cornbleet *J.A.M.A.*, 138 1150 1948.
- 12 Hinshaw *et al* stressed their viewpoint that "treatment with antibiotic should be postponed or denied to those tuberculous patients who are making satisfactory progress and who are likely to achieve the arrest of their disease as a result of conventional therapeutic methods." (Page 7²², *J.A.M.A.*, Nov 30 1946)
 - 13 *J.A.M.A.*, 137 363 1948. We may however, note that this case is not convincing because of the short duration of observation Patients must be free from the symptoms at least for two or three years after the treatment.
 - 14 Viable brucellas were constantly cultured from the livers of the infected embryos
 - 15 This technique was first developed by Goodpasture and Anderson and some sulfa compounds were tested. See *Am J Path* 13 149 1937)
 - 16 Also in spondylitis. See also the work of Herrel and Nichols, *Proc Staff Meet*, Mayo Clinic, 20-419 1949
 - 17 *J.A.M.A.*, 193 80 1949. However Spink *et al* using the same combined therapy observed only slight toxic reactions. *J.A.M.A.*, 139 35*, 1949.
 - 18 Streptomycin has proven of some effectiveness in the cases of septicemia due to *Shig dysenteriae*, *Ps aeruginosa* and *Aerobacter aerogenes* (See Herrell & Nicols also Keefer *et al*)
 19. The drug is recommended also in the treatment of lung abscesses caused by *Kleb pneumoniae* and other microorganisms susceptible to streptomycin A combined therapy of penicillin and streptomycin is recommended in such cases.
 - 20 See also the reports of Cohen and Lasser *J.A.M.A.*, 131 1126 1946 and by Kurshan and Foshay *J.A.M.A.* 131 1493 1946
 - 21 *Lancet*, January 3 1948
 - 22 When peritonitis is generalized some investigators recommend supplementing the intramuscular treatment with intraabdominal injections of streptomycin
 - 23 They gave streptomycin every three hours by mouth. See *J Clinical Invest.*, 24 898 1945.
 - 24 Thomas "Scutmas in Conjunction with Streptomycin Therapy" *Arch of Ophthalmology* 143 1439 1949

CHAPTER 11

- 1 Gardner and Chain have made a very frank admission "When compared with penicillin proactinomycin has the great disadvantage of being far more toxic."
- 2 The common bacteria like *Staphylococcus aureus* *Streptococcus viridans* and others are quite resistant to streptothricin. See Wakaman and Woodruff *Proc. Soc Exp Biol. & Med.*, 29 207 1942 also Waksman *J Bact.*, 46 299 1943.
- 3 No protection was obtained against *Staphylococcus aureus* and pneumococci See Robinson *et al.* *Science*, 99 540 1944
- 4 In the experiments on guinea pigs. See Metzger *et al.* *Proc Soc Exp Biol. & Med.*, 51 231 1942

- 5 The work of Gratia and Dath
- 6 Lesions were found in the small intestines of the mice and in some instances signs of gangrene were present in whole intestinal tract. See Rake *et al*, *Am J Med Sci.*, 210 61 1944
- 7 A provisional empirical formula was offered by Fried and Wintersteiner as $C_{12}H_{10}O_7N_2S_2Cr$. See *Science*, 101 613 1945

CHAPTER 12

- 1 According to Gordon Martin and Syngé (*Biochem J*, 37 86 313 1943) there are 24 amino-acid residues in gramicidin. The total nitrogen and oxygen content is accounted for mostly by amino acids. Some of these amino acids were identified as tryptophan glycine leucine and valine. The melting point of gramicidin is at about $+0.8\ 230^\circ\text{C}$.
- 2 Herrel and Hellman (*J Clin Invest*, 20 583 1941) have demonstrated that as small a dose of gramicidin as one microgram per cubic centimeter was able to inhibit the growth of pneumococci and a slightly larger amount arrested the multiplication of streptococci
- 3 Tyrothricin inhibits the growth and the mobility of *T buccalis* and *T mucosum* and many other vibrioform bacilli often present in the mouth (See the work of F G Johnson *J Amer Dent Assoc* 30 1909 1943)
- 4 It seems that tyrocidine inhibits cellular metabolism while gramicidin actually is highly hemolytic. A dose of 0.3-0.4 gram per kilogram of weight of the animal produces pathological changes in the spleen kidneys and other organs (See MacLeod *et al Proc Soc Exp Biol & Med* 43 461 1940)
- 5 See "Penicillin Therapy" by John A Kolmer (New York D Appleton Century Co 1947) page 397
- 6 L. N Rankin has reported five favorable cases of chronic ulcer treated with tyrothricin (*Am J Surg*, 65 391 1941) See also *JAMA* 122 760 1943 the article by Dr O Alpins.
- 7 Also in pneumococcal keratitis and blepharitis. See P Heath's article in *JAMA*, 124 152 1944
- 8 Mostly in postoperative treatment.
- 9 As a result of the disastrous effect of tyrothricin application Otenasek and Fairman initiated similar experiments on dogs. Of thirty five dogs in whom one cubic centimeter of 1:1,000 suspension of tyrothricin was injected into the cisterna magna eighteen died either immediately or within a few hours. Those with a survival period of a few hours showed massive swelling of the medulla oblongata at the site of injection. Autopsy has shown thickening of the leptomeninges with or without hydrocephalitis. The toxicity of the drug was proved experimentally as far as this type of treatment was concerned.
- 10 A thermophile *Bacillus brevis* type. Gramicidin S protects rats against the gas bacillus infection. See Sergiev *Lancet* 2 717 1944
- 11 The melting point of gramicidin is about $268\ 270^\circ\text{C}$. much higher than the melting point of the gramicidin of Dubos which is about 222°C .

CHAPTER 13

- 1 Bacitracin is now becoming available in sufficient quantities for the medical profession. The clinical trial with this drug was conducted in a number of cities such as New York, Baltimore and Philadelphia but the reports on these investigations was not available at the time the book was written.
- 2 Meleney and Johnson have given a detailed description of the method they used to evaluate the therapeutic action of the drug. See *J.A.M.A.*, 153:675, 1947.
- 3 Meleney and Johnson reported a number of other similar cases treated with bacitracin.
- 4 See Jansen and Hirschmann *Arch. Biochem.*, 4:297, 1944.
- 5 Long before Meleney and Johnson discovered bacitracin workers at the Pasteur Institute had disclosed the antibiotic properties of *Bacillus subtilis*. As early as in 1926 L. Rosenthal published a paper on this subject. Van Canneyt has confirmed some of his observations. It was found that *B. subtilis* is antagonistic not only to some Gram positive bacteria but also to some virus organisms. It seems that this bacillus might have some inhibiting activity on some saprophytic fungi. See *Comp. Rend. soc. biol.* 9: 78, 1926. *Ibid.* 95:8-8, 1926. *Canad. J. Res.*, 20:169, 1942.
- 6 Salle and Jann found that subtilin was found to be active chiefly against Gram positive bacteria. Two notable exceptions to the rule were *Neisseria catarrhalis* and *V. gonorrhoeae* both Gram negative.
- 7 Anderson and his associates limited their investigation chiefly to experiments in vitro. See *Science*, 103:419, 1946.
- 8 They tested the activity of the *Bacillus polymyxa* against *Brucella bronchiseptica* and *Escherichia coli*.
- 9 Sidney Ross et al. *J.A.M.A.* 143:1479, 1950.

CHAPTER 14

- 1 Dr. Hans Zinsser makes a special point of this characteristic of the rickettsiae. He writes: "Whether the cultivable extracellular organisms obtained from various insects not possessing pathogenicity are true rickettsiae or not we cannot state with confidence. But as far as the pathogenic rickettsiae are concerned the only multiplication that has been obtained outside the animal body has been in tissue cultures of various kinds." (Page 635, *Bacteriology*.)
- 2 Brill's disease is a mild form of typhus fever. Nevertheless according to Dr. Schoenbach "the course of the disease appeared to be definitely influenced by aureomycin therapy." *J.A.M.A.* 139:450, 1949.
- 3 M. Finland et al. *Am. J. Med.* 8:21, 1950. Some of the patients described had bronchopulmonary involvement but the others did not and it is not clear whether the response resulted from suppression of infection virus or from secondary bacterial invaders.
- 4 We may remark that agglutinins for *Streptococcus MG* often develop in the serum of patients during the course of this disease. See: *J.A.M.A.*, 139:475, 1949.

5. Wright *et al* believe that there are more than one strain of virus of *lymphogranuloma venereum*. They stated: "On the basis of our past experience we believe that in 25 cases of lymphogranuloma at this hospital there were multiple strains of the virus. There is therefore the possibility, that the effective antibiotic activity of aureomycin is not limited to a single strain. Aureomycin, a new antibiotic, with apparent virucidal properties has been used in human beings for the first time. The clinical results showed such results as to warrant further extensive research and clinical trial of the antibiotic. See *JAMA*, 138 112 1948
6. The antibiotic appears to be effective in cases of Mooren's ulcer and atypical Mooren's ulcer of known cause and it has some effect in epidemic keratoconjunctivitis. It is of value in dendritic keratitis. See Braley and Sanders, *JAMA*, 138 126 1948. Spink *et al* recently arrived at the conclusion "that the antibiotic aureomycin is a more satisfactory therapeutic agent (in brucellosis) than the combination of streptomycin and sulfadiazine and is now recommended for therapy in proved cases" *JAMA*, 138 1145 1948
7. Theodore E. Woodward *et al*. Aureomycin in Treatment of Experimental and Human Tularemia. *JAMA*, 139 830 1949
8. E. Weis and B. J. Winston *Illinois Medical Journal* 98 9 1950. The authors feel that aureomycin should be given further clinical trial in a larger series of cases so that its clinical effectiveness in poliomyelitis can be further evaluated.
9. Agar streak cultures of this organism were found to inhibit *B. subtilis*, *S. aureus*, *B. abortus*, *Esch. coli*, *A. leob. pneumoniae*, *Sal. schott* and *Shig. paradyenteriae*. See Ehrlich, Bartz, Smith, Joslyn and Burkholder, *Science*, 106 418 1947
10. The embryos were infected with *R. orientalis*, *R. prowazeki* and *R. mooseri*. The experiment was not completed. See Smadel and Jackson *Science*, 106 418 1947
11. With a 2.5 mg intraperitoneal dose the treatment could be delayed for five to eight days and still be equally effective.
12. Approximately 10 per cent of the total amount thus given was recovered in active form from the urine. See Ley, Smadel and Crocker *Proc Soc Exp Biol & Med*, 68 9 1948 and 68 12 1948
13. Ernest G. King *et al.*, *JAMA*, 143 1 May 1950. William M. Kirby (*JAMA*, 144 235 1950) remarks: "Tetramycin has been investigated clinically for less than a year and statements concerning it must therefore be accepted more tentatively than for the other two agents. From both pharmacologic and therapeutic standpoints tetramycin may be said to resemble aureomycin more closely than chloramphenicol."

CHAPTER 15

1. Pyocyanin is also considered as a pigment.
2. According to Lawrence the more soluble monophenyl derivatives are active. See *Proc Soc Exp Biol & Med*, 45 92 1940
3. R. D. Hotchkiss believes that penicillin affects the way in which bacteria use energy rather than the processes by which energy is liberated. See *Advances in Enzymology*, 4 135 1944

- 4 Autolysins might play some role in the lysis of the bacteria by penicillin. See E. W. Todd, *Lancet*, 1 74 1945
- 5 W. S. Miller *et al.*, *Nature*, 153 155 1945.
- 6 I. M. Klotz "The Mode of Action of Sulfonamides." *J Am Chem Soc.*, March 1944 p 459. Klotz concludes that "the inhibition of bacterial growth by sulfonamides may be accounted for quantitatively by assuming that the action is due to a reversible combination between the basic form of the drug and the neutral form of the protein and that the law of mass action is applicable. Equations may be derived which relate potency to the acid ionization constant of the sulfonamide and to the pH of the solution. The reversal of sulfonamide bacteriostatic by addition of p-aminobenzoic acid may be considered from the same point of view. Expressions may be obtained which account for variations in the ratio of sulfonamide to p-aminobenzoic acid from drug to drug and from one pH to another"
- 7 Among the antibiotics extracted from plants are *allicin* from *Allium sativum* and *tillandsianin* from *Spanish Moss (Tillandsia usneoides)*, and many others. Recently a new antibiotic was described named *entriptide* which will protect cattle from African sleeping disease. It is claimed
- 8 Agranulocytosis is due to a depression of the bone marrow by sulfonamides. Penicillin is effective in treatment of this condition.
9. No data on this subject are as yet available.
- 10 Hematuria and functional impairment of the kidneys are infrequent in the treatment with sulfanilamide but quite frequent in sulfadiazine therapy
- 11 These reactions might be of considerable gravity and associated with neurological symptoms in mild form.
- 12 Miller *et al* found a great variation in the susceptibility of bacteria of the same species. They reported that the natural resistance of six strains of gonococci vary from seven to forty units. They observed an even more striking variation among the meningococci. See *J.A.M.A.*, 150 485 1946
- 13 Streptococcal and pneumococcal infections seem to respond better to penicillin therapy than staphylococcal endocarditis.

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